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5570

The Excretion of Vitamin B by Artificially Fed Infants.*

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Vitamin B in the urine and feces of animals and in human urine has been demonstrated by Muckenfuss,[1] Van Der Walle,[2] and Cooper.[3] According to Cowgill[4] the vitamin in the urine represents the amount ingested in excess of the needs of the animal, whereas that in the fecal excretion has been shown to be unabsorbed material.[5] The presence of Vitamin B in the urine of an animal, therefore, would seem to indicate the adequacy of the diet as a source of this factor; and the amount of the vitamin excreted in the feces should give some measure of the amount not available to the organism.

With such an interpretation in mind, a study of the intake and

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* This investigation was aided by a fellowship grant from the Mead Johnson Company of Evansville, Ind.

[1] Muckenfuss, A. M., *J. Am. Chem. Soc.*, 1918, **40**, 1606.

[2] Van Der Walle, N., *Biochem, J.*, 1922, **16**, 713.

[3] Cooper, E. A., *J. Hyg.*, 1914, **14**, 12.

[4] Cowgill, G. R., Deuel, H. J., and Smith, A. H., *Am. J. Physiol.*, 1925, **73**, 106.

[5] Dutcher, R. A., and Francis, E., Proc. Soc. Exp. Biol. and Med., 1923-24, **21**, 189; McCollum, E. V., Simmonds, N., and Becker, J. E., *J. Biol. Chem.*, 1925, **63**, 547; Salmon, W. D., *J. Biol. Chem.*, 1925, **65**, 457; Steenbock, H., Sell, M. T., and Nelson, E. M., *J. Biol. Chem.*, 1923, **55**, 399.

excretion of Vitamin B (the antineuritic factor) has been made on a group of 11 artificially fed infants and the retention of the vitamin determined. Thirty-three collections of urine and feces were tested. The food of all the children consisted essentially of cow's milk, a carbohydrate modifier free from Vitamin B, orange juice, and cod liver oil. In a few cases a Vitamin B containing extract was added to the feeding mixture in order to ascertain the influence of a larger ingestion. The determination of the amounts of Vitamin B in the ingested food and in the excreta was made by a biological assay on young rats of uniform weight and uniformly depleted of their vitamin stores. Each sample of the concentrated urine and dried feces was fed at various levels, from one to 6 rats being used at each level. Parallel experiments with yeast indicated no destruction of Vitamin B by the methods of concentrating and drying employed. A unit of Vitamin B was arbitrarily defined for the purpose of this study as the amount of the vitamin necessary to cause a young depleted rat weighing about 60 gm. to gain one to 2 gm. per day during the experimental period of 8 days.

Vitamin B, from 13 to 40 units, in the urine of the infants seems to indicate the adequacy of the cow's milk feeding mixtures tested as sources of this factor. The amounts of milk fed were comparable in some instances with the amounts usually recommended by pediatricians, 1.5 oz. per pound per day. With infants younger than 3 months, however, the milk ingestion was somewhat higher. The amount of Vitamin B retained per unit of weight (av. 8.4 units per kg.) is fairly consistent for all the infants whose vitamin balances have been calculated. The addition of 12 units of Vitamin B to the formula in the form of 30 cc. of wheat germ extract produced no significant change in the amount of the vitamin retained. The excretion of large amounts of Vitamin B in the feces was observed to be coexistent with a fatty stool. In general, the amounts of milk necessary to furnish Vitamin B for growth and to allow for losses through lack of absorption in the infants studied were found to be comparable with the amounts commonly prescribed for artificially fed infants, namely 1.5 oz. per pound of body weight. With the younger baby slightly larger amounts of milk would seem desirable. The data suggest that the Vitamin B requirement per unit of body weight is higher in early infancy and decreases with age.

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The Micro Determination of Citric Acid by the Thunberg Methylene Blue Method.

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This method[1] is based on the capacity of an enzyme found in cucumber seed to liberate hydrogen from citric acid for the reduction of methylene blue to the leuco compound. The speed of decolorization varies with the amount of citrate up to a certain concentration. Beyond this "critical" concentration there is no further decrease in decolorization time.

The least amount of citrate necessary for maximum speed of decolorization is influenced by the nature of the enzyme extract; this depends on the source of the cucumber seed, the amount of grinding and centrifuging in its preparation, the H ion concentration (during extraction and the reaction proper) and on the time the extract stands before use. Enzyme extracts less than 40 minutes old rapidly decolorize Me-blue even in the absence of citrate; with increasing age of the extract the decolorization time is greatly prolonged unless citrate is present. In practice enzyme extracts about 2½ hours old have proved most satisfactory in revealing the "critical" citrate concentration. Of 20 determinations made on standard freshly prepared citrate solutions 15 were within 6% of the average. The other 5 varied more widely perhaps because of slight irregularities in the preparation of the enzyme extract. Adams and Boothby[2] suggested extraction of the seed with water instead of with 0.87% K_2HPO_4 at pH 8.2 because water extracts contain less extraneous substances which have an accelerating effect like that of citrate. The advantage gained by this procedure is somewhat offset by the higher "critical" concentration of citrate necessary, and by the absence of buffer, other than that supplied by the seed.

The sensitivity of the method is directly proportional to the amount of methylene blue present in the reaction tube. Large amounts of methylene blue (0.3 cc. of 1:30,000 solution) permit accurate reading; small amounts (1:120,000) are difficult to read but allow the estimation of as small amounts of citrate as 0.2 mg. per 100 cc.

The modification of the method for the determination of citric acid in the blood suggested by Benni, Schersten, and Östberg[3] gives low and unreliable results because it compares a tube containing a high concentration of Na and Ca with one in which no salts are present and without reference to the "critical" point. The presence of Na, Ca, or hexose di-phosphate alters the time required for decolorization but does not change the least amount of citrate necessary for maximum speed of decolorization. By increasing the sensitivity of the method through use of a smaller concentration of methylene blue, the effect of these substances is avoided.

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[1] Thunberg, T., *Biochem. Z.*, 1929, **206**, 109.

[2] Adams and Boothby, as reported at Montreal meeting, Am. Soc. Biol. Chem.

Oxalic acid in concentrations 3 times that required to prevent coagulation increases the amount of citrate necessary for maximum speed of decolorization. Oxalates therefore can not be used as anticoagulants if plasma citrate is to be determined by this method.

Preliminary experiments on rabbits showed that the citric acid content of the serum dropped very markedly during a 3-day fast, and that the administration of $NaHCO_3$ to fasting rabbits increased the serum citrate.

5572

The Psychopathological Effect of Sodium Amytal.

ERICH LINDEMANN. (Introduced by Lee E. Travis.)

From the State University of Iowa, Psychopathic Hospital, Iowa City.

Isoamylethylbarbiturate was introduced into psychiatry by Lorenz and Bleckwenn in 1929. It was recommended to produce profound sleep which in certain neuropsychiatric patients was followed by peculiar changes in behavior. Lucid intervals with good contact and almost complete insight were produced in certain cases of catatonic dementia precox. Depressed or excited patients had periods of calmness and contentment. In some cases, improvement, in others a rapid recovery followed the repeated induction of sodium amytal narcosis.

We conducted experiments guided by the assumption that the striking changes following sodium amytal administration cannot be sufficiently explained on the basis of the narcosis produced. We studied the drug action, therefore, in very small doses not leading to any narcosis or sleep at all. In examining patients' responses as compared with the observations by the authors mentioned above we found that after the injection of less than half of the dose used by

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[3] Benni, Schersten, and Östberg, *Biochem. Z.*, 1930, **223**, 443.

Bleckwenn, when no signs of narcosis could be observed, there nevertheless was a striking change in the patient's behavior similar to the change described as the result of narcosis. This led us to a more systematic investigation of the effect of sodium amytal, particularly since such small doses are void of all the disadvantages and dangers involved in any prolonged narcosis.

It seemed indispensable to study the effect of the same doses as used in psychotic patients with normal individuals. Sodium amytal crystals dissolved in distilled water to a 1 gr./1 cc. solution were injected intravenously. One grain was injected during the period of a minute. The injection was stopped as soon as the patient showed the first signs of psychological change. The administration of 3 to 4½ grains was sufficient to produce the change which we wanted to study. Twenty-four patients and 4 normal individuals were used as subjects. The normal individuals were one staff member of our hospital and 3 graduate students accustomed to making introspections. In all subjects we found certain neurological changes, such as speech defects of the paretic type, nystagmus, a disturbance of eye muscle coordination, and a mild degree of ataxia. There was also an increase in the threshold for pain stimuli as well as for smell and taste stimulation. There was the objective appearance of mild fatigue without any subjective signs of it.

The psychological changes in normal individuals were characterized by a feeling of serenity and well-being, a desire to communicate and to speak about problems of personal matters usually not spoken of to strangers. There was also the feeling of being unable to guard against saying things which one does not want to and an inability to refuse to answer questions even if they refer to very intimate matters. There was a feeling of increased physical and mental abilities but no objective increase in psychomotor activities.

The subject's objective behavior was friendly, amiable, emotionally warm, sometimes sentimental, combined with a certain distractibility and lack of power or willingness to stick to the subject of conversation. As a whole, the picture was of mild euphoria with rapid flow of associations but no increase in psychomotor activities.

The patients belonged to the schizophrenic, cyclothymic, and psychoneurotic group. The outstanding change was an increase in ability or willingness to communicate thought content and memories. Catatonic patients who had been mute for months became communicative and gave valuable material about their trends of thought. Depressed patients told about subjective reasons for their feelings of guilt. The psychomotor activity in psychotic patients showed a marked change in the direction of metrokinetic behavior. Catatonic patients lost their rigidity and their cataleptic symptoms. Patients with agitated depressions became quiet. Hyperkinetic depressions were changed to normal states of motor activity.

The mood appeared indifferent or mildly euphoric. Anxiety and apprehension gave place to mild indifference with a tendency to joke. In no case did we find a change in the essential structure of the systems of delusions and of perceptive distortions.

Our studies show that sleep and narcosis are not necessary conditions for the production of the mental changes discovered by previous authors as the result of sodium amytal injection. In normal individuals the drug produces a mild euphoria and a release in inhibitions and reserve which usually prohibit the individual from communicating about matters of emotional significance. The same mechanism seems to be at work in psychotic patients where it makes possible communications of the thought content, inaccessible to the usual means of clinical approach. The value of the method described above for diagnosis and for psychopathological investigation of psychiatric conditions seems to be established. The effect of sodium amytal in this respect is similar to that of cocaine which was used by Berger in Germany. Amytal is to be preferred because it does not seem to have any undesired concomitant effects. The therapeutic influence of the drug does not seem to be specific. It allows a period of emotional rapport with the patient and gives access to thought material which can be utilized for subsequent psychotherapeutic efforts.

5573

Exchange of Oestrin and Corpus Luteum Hormones in Parabiotic Female Rats.*

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From the Zoological Laboratory, State University of Iowa.

In a previous paper the author has described a method for testing the amount of blood exchange in parabiotic rats.[1] A large series of twins show that an average of 24% of dye is found in the uninjected animal at the end of one hour. Normal parabiotic female rats thus tested for their vascular connections come to maturity independent of each other. There is sufficient oestrus producing hormone exchanged to disturb slightly the cycles of each twin member, but not enough to synchronize them. The finer individual differences of each pair, according to a higher or lower amount of blood exchange are still under investigation.

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Hill, Robert T., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 592.

In a series of 11 pairs in which the oestrus cycles had been followed over a long period, both ovaries were removed from one female. The castrate subsequently goes into anoestrum and only occasionally shows signs of oestrus while the normal female co-twin has normal cycles. This agrees with the above mentioned fact that there is little interference of oestrus in pairs of normal females. Our results vary from those of Martins,[2] who describes the production of oestrus in a castrate member by carry-over of hormones from the normal co-twin. The rats he used were put in parabiosis with the body cavities joined (coelioanastomosis). In this condition it is possible that some hormone exchange may take place also through the medium of the peritoneal fluids and not exclusively through the blood. To check this we have followed the cycles of 9 pairs, one member a castrate, in which the body cavities were not connected, and of 2 pairs in which they were in open connection. At present no difference has been noted between the 2 conditions.

At variance with the results of Zacherl[3] we have found that during pregnancy of one mate the oestrus cycles of the non-pregnant twin are from the beginning almost entirely inhibited by the action of the corpus luteum hormone of the pregnant member. In groups IX and XX fertilization occurred in the left member. Oestrus nearly ceases in both members of each pair after the start of pregnancy until a few days after parturition, when normal oestrus returns in the absence of nursing young. The curves of groups XXI and XXII show the consequences of the effect of a period of lactation following parturition. As usual the lactating female stays in dioestrum. After parturition in group XXI the non-pregnant member started oestrus, but did not show the normal rhythm. Parturition in group XXII was followed by onset of normal oestrus in the non-pregnant co-twin. Stopping of lactation is followed by normal oestrus in both members of a pair.

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[2] Martins, Thales., *Compt. Rend. Soc. Biol.*, 1929, **102**, 605, 614.

[3] Zacherl, H., *Klin. Wochenschrift.*, 1927, **6**, 1614.

The above facts reveal a striking difference between the oestrus and corpus luteum hormones. It may be explained in 2 ways. Either the corpus luteum hormone is secreted in a greater surplus than oestrin, or it may be absorbed more slowly, which would allow for a more nearly even distribution between both members of a pair.

Furthermore, the hormones transferred during pregnancy from the pregnant member stimulate partial hypertrophy of the mammary glands of the non-pregnant co-twin.

5574

Soil Aeration as a Factor in Growth and Root Development of Plants.

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From the Department of Botany, State University of Iowa.

The soil in which certain aquatic and terrestial angiosperms were growing was slowly aerated daily for a period of one-half to 5 hours by means of compressed air supplied through perforated coils imbedded 8 inches below the surface. *Helianthus annuus, Triticum vulgare, Glycine soja, Linum usitatissimum* and cuttings of *Vitis vulpina* were grown in sand and in loam. At the age of 2 months conspicuous differences were apparent in aerated and unaerated plants on the same soil as well as between plants similarly treated but in different soils. As might be anticipated aeration did not produce the same effects in plants grown in loam and in sandy soil. Structural differences occurred in both tops and root systems, but especially the latter.

In general, roots in the aerated soils were distinctly fibrous in character, more numerous and longer, forming branches of secondary and tertiary rank. Total surface in aerated roots was twice or more that of control roots, but the root hair zone was smaller. Fewer hairs developed and these in turn were not as long-lived in aerated plants as in the controls due to the more rapid elongation of the aerated roots. Vascular elements a few inches above the root hair zone were less developed in aerated roots.

The striking effect of aeration on tops was acceleration of growth in early stages, attributable to increased length of basal internodes rather than to an increase in the number. Contrast in internodal distance diminished noticeably, however, above the median nodes and there was little or no difference in size of internodes near the tips. Elongation continued in controls after aerated plants had started to flower. This response tended to reduce size differences when mature plants of both groups were compared. The physiological effect of aeration on tops appeared to be acceleration in development with earlier maturation rather than prolonged development and increase in bulk. Percentage dry weight of top was the same in tops of aerated and unaerated plants.

B. E. Dean, working in this laboratory under the author's direction, has found that root systems of submerged aquatics, such as Typha, Sagittaria, and Hibiscus increase greatly in size in aerated sand, clay and muck. Roots in aerated soils were longer, more highly differentiated as shown by marked lignification of the new primary roots in many cases. In the main, unaerated roots were fibrous but less numerous than the corresponding fibrous elements of aerated root systems. Submerged (but not subterranean) water roots in Typha and Sagittaria were more numerous and profusely branched in unaerated soils. In every case larger tops were associated with more extensive root systems.

5575

Exchange of Hypophysis Hormones in Parabiotic Amphibians.*

EMIL WITSCHI.

From the Zoological Laboratory, State University of Iowa.

Experiments of Burns[1] and the author[2] on gonad development in parabiotic amphibians gave evidence of interesting variations in the mode of hormonic response in heterosexual combinations, which partly, are due to different hormone concentrations. Unfortunately the development of these reactions can not be followed satisfactorily in live pairs. In this respect conditions are much more favorable in the study of the exchange of hormones of the hypophysis, because of the marked external effects which they produce as controllers of metamorphosis and chromatophore reaction.

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Burns, R. K., *J. Exp. Zool.*, 1930, **55**, 123.

[2] Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 763; *J. Exp. Zool.*, 1931, **58**, 113; Witschi and McCurdy, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 655.

The Californian newt, *Triturus torosus,* ordinarily metamorphoses at about 5 months old. After hypophysectomy it becomes a perennibranchiate. As in other amphibians complete hypophysectomy causes also the contraction of the epidermal melanophores. The incomplete operation, however, leaves either the color unchanged or the larva becomes very dark. In the latter case the persisting intermediate lobe which, according to Allen,[3] controls the melanophore reaction, seems to hypertrophy.

Parabiotic twins of this newt likewise metamorphose when 5 months old. If hypophysectomized even as late as at the end of the fourth or beginning of the fifth month, they become perennibranchiate and eventually also albinistic. If, again on the verge of metamorphosis, one only of the pair is hypophysectomized, metamorphosis is delayed for from 2 to 6 months. From the fact that the unoperated twin-mate needs so much time to restore the hormone concentration to the level required for the inauguration of metamorphosis, we conclude that it can accomplish this not simply by an increased physiological activity but only through some hypertrophy of its hypophysis. It becomes evident also that the anterior lobe normally does not release an overdose but just the adequate amount of the metamorphosis hormone. Thirdly, we note that this hormone is practically evenly distributed in the twin pair, for when metamorphosis after this delay comes on, it begins simultaneously in both animals.

Slightly different is the melanophore reaction. If the hypophysis is removed from one member of a Triturus pair, it turns distinctly lighter than its co-twin and this effect is lasting even through metamorphosis.

This difference between the metamorphosis and the melanophore reaction becomes even more pronounced in parabiotic chains. In this mode of grafting the hypophysis as a rule is completely removed from the posterior animal. Consequently its melanophores are contracted from their very first appearance and stay so permanently. If the head is cut off the first member, its melanophores contract also, within a few hours.

One may, however, by cutting off the dorsal front only of the second animal, preserve part at least of its hypophysis. In such chains both animals have expanded melanophores. Hypophysectomy of the first animal of such a chain made it turn much lighter because of melanophore contraction, within a few hours. This pair has never metamorphosed but died at the age of 18 months. Serial sections show a minute fragment of hypophysis, probably intermediate lobe, in the first one, not over 1/20 of the total gland. In the second one the Diencephalon has been pushed away by the spinal cord of the first animal, connecting with the Rhombencephalon of the second one. The infundibular region is covered with an extensive crust of hypophyseal lobules which, structurally, resemble rather the intermediate lobe type.

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[3] Allen, B. M., *Anat. Rec.*, 1925, **31**, 302.

Again the color differences in these chains are lasting, even through metamorphosis. And again do we observe that the metamorphic changes of both animals run synchronously.

It appears that the zone of fusion acts like a filter, allowing the metamorphosis hormone to pass but retaining the melanophore hormone. Microscopic study shows that the circulatory systems of the 2 individuals are connected by a net of capillaries only. This seems to indicate that the melanophore hormone is quickly used up, disappearing in the capillaries.

If frogs or toads are grafted in chains the hypophysis deprived animal stays lighter for about the first 2 weeks. In chains of 3 the third animal is very light for an even longer period. As in twins, however, the second animal later turns to its normal color. Metamorphosis occurs simultaneously. When the resorption of the tail stump of the first animal begins, the second animal presently turns light again.

Frogs and newts therefore give identical results for the metamorphosis hormone and are but quantitatively different with respect to the melanophore hormone.

5576

The Barrier Between the Blood and Cerebrospinal Fluid; a Microchemical Modification of the Walter Method.

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From the Iowa Psychopathic Hospital, State University of Iowa.

In investigations of this barrier by the Walter bromide method,[1, 2, 3, 4, 5] the value of the procedure as an aid in the diagnosis and

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[1] Malamud, Wm., Fuchs, D. M., and Malamud, N., *Arch. Neurol. and Psychiat.*, 1928, **20**, 780.

[2] Malamud, Wm., Wilson, R. B., *Arch. Neurol. and Psychiat.*, 1929, **22**, 1135.

[3] Malamud, Wm., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 477.

[4] Malamud, Wm., Rothschild, D., *Arch. Neurol. and Psychiat.*, 1930, **24**, 348.

[5] Malamud, Wm., Hayward, E. P., *Zeitsch. ges. Neurol. und Psychiat.*, 1930, **128**, 295.

the control of treatment of mental diseases was demonstrated. The practical application of the method, however, was seriously hampered by technical drawbacks, one being the comparatively large amount of spinal fluid (8 cc.) necessary for the performance of the test. Such quantity at one puncture is, with few exceptions, contraindicated in most cases of disease of the central nervous system. In most instances it may be followed by headache, vomiting, backache, etc. In some diseases (such as intracranial tumors) it is quite dangerous and in some cases followed by death. The removal of small quantities is practically never dangerous if carried out with a reasonable degree of care. We have therefore searched for a modification of the original method that would enable us to carry out the test with a smaller quantity of fluid.

We here describe such a modification and its application in 119 cases where both methods were used and checked against each other, and where the large quantity necessary for both determinations could be taken without any untoward sequelae.

After administration of bromides and preparation of patient,[1] 15 cc. of venous blood is taken and treated with trichloracetic acid and gold chloride.[1] The solution is then read in a colorimeter against a standard of known bromide content, and the bromide concentration in the blood computed.

Three cc. of spinal fluid is then obtained by lumbar puncture and to this 0.6 cc. of 30% trichloracetic acid is added. After allowing this to stand for 15 minutes, it is filtered and to 2 cc. of the filtrate 0.4 cc. of 0.5% gold chloride is added. The solution obtained is then read against a standard bromide solution and thus the bromide content of the spinal fluid is determined. By dividing the bromide content of the blood by that of the spinal fluid we get the *distribution ratio*. (To gain time one can read the spinal fluid directly against the blood and thus obtain the ratio.) The colorimetric determinations are made with the aid of a Duboscq microcolorimeter No. 3625 instead of the original macrocolorimeter No. 2502.

In 119 cases there was a very high degree of correlation between the readings obtained by the 2 methods. In a large number of them the readings were actually identical. In the others they varied within 0.07. Since the variation in different readings by the same method and the same person are at least at high as these, it would seem that there is practically no difference between the two.

The correlation between the 2 methods, then is +.999±.0001.

The results obtained show that the above described modification is reliable and can be safely used instead of the original method.

Missouri Section.

St. Louis University School of Medicine, May 13, 1931.

5577

The Luteinizing Substance of Pregnancy Urine.

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From the Laboratory of Biological Chemistry, St. Louis University School of Medicine.

That pregnancy urine contains a luteinizing substance was first shown by Zondek.[1] From his later work[2] it appears that the luteinizing substance is different from the agent that causes ripening of the follicles, even though such a separation has not been made with urines of pregnancy.

Extracts prepared by us from pregnancy urine seem to produce both maturation of follicles and luteinization. In the immature mouse or rat premature estrus is effected by their administration. We have tentatively defined one mouse unit as the minimum amount that causes opening of the vagina and estrus on the twenty-second to twenty-fourth day of age, after being administered subcutaneously to 19-day-old mice in 6 equal portions during the course of 3 days. This criterion of activity seems to be quite satisfactory if one is assured of the absence of the estrogenic substances. Depending upon the degree of purification, the mouse unit has varied from 0.03 to 0.001 mgm. This amount produces a marked hyperemia and development of the ovaries and uterus but very little luteinization.

The daily administration of these extracts to adult female rats produces for the first 5 to 10 days an almost continuous state of estrus, due probably to stimulation of the follicular apparatus. Cornified cells then disappear from the smears, possibly as an effect of the extensive luteinization. The inhibition of estrus persists for 5 to 12 days following the cessation of the injections, the cycles then reappearing with a slight prolongation of the diestrus interval. The ovaries of the treated animals are loaded with corpora lutea and weigh 4 or 5 times as much as those of the controls.

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[1] Zondek, B., *Klin. Wschr.*, 1928, **7**, 30.

[2] Zondek, B., *Klin. Wschr.*, 1930, **9**, 1207.

Kelly[3] has reported that the injection of pregnancy urine into pregnant guinea pigs invariably produces abortion. The administration of our extracts to rats greatly prolongs the gestation period.[4] This is true when the injections are started at any time between the fourteenth and nineteenth days of pregnancy. At autopsy the fetuses are usually dead and partially resorbed but fully developed and often of greater than normal size. The ovaries are much larger and more luteinized than those of control pregnant rats.

Since the luteinization is so pronounced it would seem reasonable to attribute the delay of parturition to this phenomenon. To test this idea we have administered the extracts to ovariectomized pregnant rats but in no case was the gestation period prolonged. Apparently, the presence of the ovary is necessary to produce this effect and since large quantities of theelin have no influence on the pregnancy, it seems probable that the corpora lutea are responsible for the retention of the fetuses.

5578

Chromatophore Reaction to an Oestrogenic Hormone.

PAUL L. CARROLL, S. J. (Introduced by E. A. Doisy.)

From the Department of Biology, St. Louis University.

Some 3000 tadpoles *(Rana pipiens)* were treated during 5 months with the oestrogenic hormone theelin obtained from the Biochemical Laboratory of Doctor Doisy. Several series of experiments were carried on simultaneously in which the tadpoles were exposed to different concentrations of theelin over varying periods of time. In other experiments 100 tadpoles were injected with the hormone.

In one series 250 tadpoles just emerged from the gelatin coating were placed in a 1000 cc. aqueous solution of theelin 0.0000003125 mg./cc. for 60 minutes. On successive days the exposure time was increased by 60 minutes for a period of 10 days. This time increment was kept constant during the rest of the experiment but the concentration of the hormone was doubled every 10 days until a concentration of 0.0000025 mg./cc. was attained. The experiment was completed on the 40th day. Higher concentrations of the hormone effected a marked change during the last 10 days of the ex-

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[3] Kelly, L. G., *Anat. Rec.*, 1931, **48**, 50 (supplement).

[4] Levin, L., Katzman, P. A., and Doisy, E. A., in press.

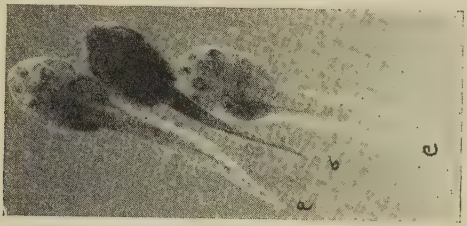

FIG. 1. ×1.5

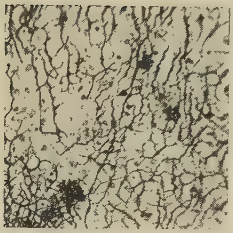

FIG. 2. ×72

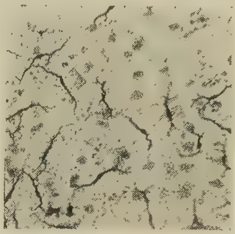

FIG. 3. ×72

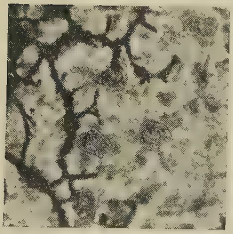

FIG. 4. ×154

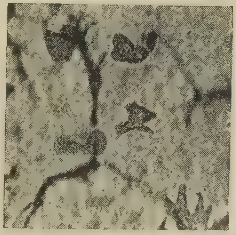

FIG. 5. ×154

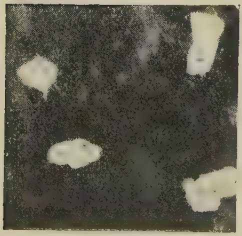

FIG. 6. ×450

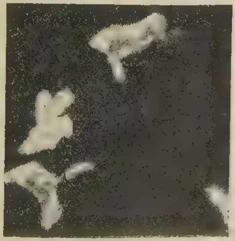

FIG. 7. ×450

periment. Microscopic examination of fresh epidermis mounts revealed a recession of pigment centrally toward the cell body in the epidermal melanophores and a pseudopodial expansion of the leucophores.

Some 65 tadpoles (total length 26 mm.) were injected intraperitoneally with 0.05 cc. theelin 0.00035 mg./cc. on 3 successive days by means of a glass needle (0.1 mm. diameter) attached to a Chambers microinjection apparatus. Color change (silvering) was first observed on the 6th day after the first injection. Tadpoles A and C (Fig. 1) show the color change following the injection. Tadpole B (Fig. 1) is a control. This is a photograph of the living animals quieted in iced water. Fig. 2 shows a mounted section of epidermis from the mid-tail region of tadpole B. The epidermal melanophores are multi-branched with frequent anastomosis. Fig. 3 shows a similar section from tadpole A. A recession of pigment centrally toward the cell body is seen in the epidermal melanophores. Practically no anastomosis nor marked branching is evident. Fig. 4 shows the normal contour of the leucophores in the control tadpole. Fig. 5 shows the pseudopodial expansion of the leucophores in the experimental animal. Figs. 6 and 7 are microphotographs taken by dark-field illumination of the leucophores from control and experimental animals respectively. The normal contour of the leucophores in the control animal and the pseudopodial contour of the leucophores of the experimental animal are evident. All of the photographs are made from untouched negatives.

Conclusions. The oestrogenic hormone apparently has an effect upon the pigmentary system of the tadpole causing albinism. The chromatophore reaction involves a centripetal movement of pigment in the epidermal melanophores and a pseudopodial expansion of the leucophores. The velocity of the reaction is directly related to the concentration of the hormone and the duration of exposure. Intraperitoneal injection causes a more rapid response. It is generally admitted that the pigmentary system of amphibia is controlled by the secretions of the pituitary. Extracts from the pituitary gland cause melanism. The oestrogenic hormone causes albinism. It is probable that in the control of the melanophores and the leucophores there exists a physiological antagonism between the secretions of the pituitary and the oestrogenic hormone. A further histological study of the effect of the injected theelin upon the amphibian pituitary will be presented in later studies.

5579

Effect of Super-Centrifuging on the Fission Rate of Paramecium.

PATRICK H. YANCEY. (Introduced by M. S. Fleisher.)

From the Department of Biology, St. Louis University, and the Marine Biological Laboratory, Woods Hole.

McClendon[1] reported that prolonged centrifuging (585 times gravity for 24 and 32 hours) increased the fission rate of paramecium. However, his experiments were extended to so few animals (6 experimental and 6 controls) and his controls divided so slowly (an average of 0.11 per day), and had such a high mortality (only one survived the whole period of the experiment) that some doubt has been cast on the validity of his conclusions.[2]

In the present investigation higher centrifugal forces (21,000 and 32,000 times gravity) were employed but for shorter periods (one to 45 minutes). The apparatus used was the Sharples Super-Centrifuge, hence the treatment is referred to as "super-centrifuging." The form experimented on was *P. multimicronucleata* obtained from Prof. L. L. Woodruff of Yale University. Over a hundred individual lines were followed but only the average division rate of each set of super-centrifuged animals is given in the table. The number of animals in each set varied from 3 to 20. The individual organisms and their controls were maintained in 0.025% beef extract solution according to the method of Woodruff and Baitsell[3] The duration of the experiments was from one to 39 days.

The results are given in Table I in which are recorded minutes of super-centrifuging, days of survival of the individual lines, average fission rate of each set of super-centrifuged animals and their controls during the first 24 hours after treatment, during the first 5 days, and during the whole period of observation when this extended beyond 5 days. The reason for this division was to ascertain whether the immediate effect of super-centrifuging might be to stimulate division as McClendon found, because the majority of his divisions occurred during the first 5 days after treatment. A further division of the experimental animals into "Normals" and "Abnormals" is made on the basis of absence or presence of visible morphological changes in them following super-centrifuging. Where the same controls were used for more than one set of experimental animals (as happened when more than one batch were super-centrifuged on the same day) they are counted only once in the averages. The values for the "Normals" and "Abnormals" are combined and the rates for the 3 periods are averaged.

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[1] McClendon, J. F., *J. Exp. Zool.*, 1909, **6**, 265.

[2] Dobell, C., *J. Gen.*, 1914, **4**, 131.

[3] Woodruff, L. L., and Baitsell, A., *J. Exp. Zool.*, 1911, **11**, 135.

TABLE I.
Fission Rate of Super-centrifuged Animals and Controls.

Minutes Super-centrif.	Days Survival	First 24 Hours			First 5 Days			Total Period of Observation		
		Norm.	Abnor.	Cont.	Norm.	Abnor.	Cont.	Norm.	Abnor.	Cont.
1	6	1.00	3.00	2.30	1.20	1.40	1.32	1.00	1.20	1.26
2	39	0.70	0.00	1.00	1.00		1.14	1.12		1.28
3	38		0.00	0.00		0.40	1.14		1.16	1.25
5	2	2.60	1.60	1.00						
10	30	3.00	1.00	1.00		1.16	1.34		1.21	1.28
15	26	1.10	1.00	2.00		1.16	1.32		1.11	1.30
20	21	3.00	2.00	1.40		1.24	1.26		1.19	1.42
25	3		0.80	1.00						
30	3		0.00	1.70						
45	6	0.00	1.00	0.60		1.84	1.02		1.79	1.10
Aver.*		1.19	0.72	1.27	0.90	1.03	1.23	1.06	1.14	1.25
Combined av.*		0.95			0.96			1.10		

* These averages represent 20 experiments of which samples only are given in the table.

From these results it appears that the effect of super-centrifuging is in general to lower slightly the fission rate of paramecium. This is true not only during the entire period of observation (77% of cases) but also during the first 24 hours (65%) and during the first 5 days after treatment (75%). This lowering of the fission rate seems to be due to an internal disorganization of the cell because it happened in both "Normals" and "Abnormals" and persisted in the latter even after all visible structural defects due to the treatment had disappeared. However, this change cannot be regarded as a mutation because the longer the lines persisted after super-centrifuging the nearer the rate approached that of the controls. It may be what Jollos[4] calls an "enduring modification", that is, one which persists for some time but not permanently.

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[4] Jollos, V., *Biol. Zentralbl.*, 1913, **33**, 222.

5580

The "Iterative" Nature of the Vagus Nerve Fibers to the Heart.

ARTHUR S. GILSON, JR. (Introduced by Joseph Erlanger.)
From Washington University School of Medicine.

If the right vagus nerve of a turtle be stimulated electrically, the degree of inhibition produced in the heart depends upon a number of factors. If we consider only those introduced by changing the nature of the stimulation used, the inhibitory effect will depend upon (1) the shape of the time-voltage curve of the shock used, (2) the strength of the individual shocks used, (3) the number of stimuli applied to the nerve, and (4) the frequency of stimulation. Since such variables must act through changes in the nerve fiber responses and since such responses follow the all-or-none law of fiber response, the following argument must hold. The strength of stimulus applied must, of course, be great enough to reach the threshold of one or more vagus inhibitory fibers before any cardiac inhibitory effects will appear. As the shock strength is increased above this threshold strength, there will be a resultant increase in the number of nerve fibers stimulated. There will also be an increased inhibitory effect upon the heart. The inhibition may attain a certain maximum which is probably achieved in consequence of the stimulation of all of the cardiac inhibitory fibers in the nerve. The effect of an increasing number of stimuli in producing an increased inhibitory effect can be thought of only as an increase in peripheral effect. The effect of changing the stimulus frequency is such that increasing the frequency of stimulation up to a certain amount (between 10 and 40 stimuli per second in most cases) causes an increased inhibitory effect upon the heart. With increasing rates of stimulation the inhibitory effects become no greater but rather become less. The decrease in effect with an increase in rate is probably related to a Wedensky type of inhibition which develops in the nerve (or the end mechanism) with too rapid stimulation.

The summating effect of repeated stimulation of the vagus and certain other nerves led Lapicque[1] to the expression of the theory of *iterative nerves*. In iterative nerves, the chronaxie values of the nerves are presumably much less than the chronaxies of the respective end organ mechanisms. A series of several nerve impulses are supposed to be necessary for building up, by the phenomenon of *latent addition*, an excitation process in the end organ sufficient for a threshold response. The vagus nerve as inhibitor to the heart is considered to be typical of this group of nerves.[2] The criterion of threshold effect which is adopted in this case by Lapicque and Meyerson is the complete stoppage of the heart.

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[1] Lapicque, L., *C. R. de l'Acad. des Sci.*, Paris, 1912, **155**, 70.

We find that in many preparations, it is possible to stop completely the entire heart of a turtle by a single shock applied to the right vagus nerve. It can be demonstrated by means of a recording instrument such as the cathode ray oscillograph that the stimulus produces but one single response volley in the nerve. The assumption of the necessity of repetitive stimulation of the nerve to produce stopping of the heart is therefore without support in fact, even if we were to accept the stopping of the heart as being a threshold effect. That peripheral summation is of importance may be recognized from any group of records such as those of Figure 1.

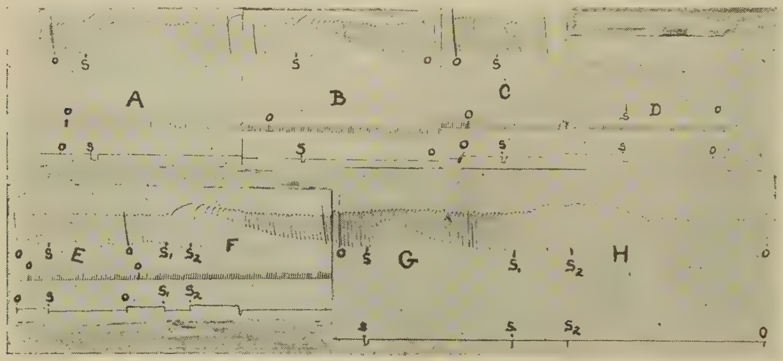

FIG. 1.

Records obtained with lever attached to one atrium of turtle *(P. elegans)*, 3/10/31.
A. 1.0 mfd., 22 volts, right atrium, right vagus, single shock.
B. 1.0 mfd., 6 ” ” ” ” ” ” ”
C. 1.0 mfd., 3 ” ” ” ” ” ” ”
D. 0.25 mfd., 6 ” ” ” ” ” ” ”
E. 0.01 mfd., 45 ” ” ” ” ” ” ”
F. 0.01 mfd., 45 ” ” ” ” ” 3 per second between S_1 and S_2.
G. 0.25 mfd., 22 ” left atrium, left vagus, single shock.
H. 0.25 mfd., 22 ” ” ” ” ” 3 stimuli per sec. between S_1 and S_2.
Time intervals, 1.0 second.
Condenser in series with 10,000 ohms. Nerve on silver wire electrodes, 5 mm. separation.

The inhibitory effects upon the heart as a result of stimulation of the vagus by electrical shocks of various forms, strength, frequency, and number have been studied in our own records and those published by previous workers in this field. The results obtained appear to be entirely consistent with the view that the amount of cardiac inhibition produced is dependent upon the number of vagus inhibitory fiber impulses delivered in a given period of time. If stopping of the heart be taken as an example, we may say that *n* vagus fiber impulses are necessary to produce this effect. Considering only shocks, each one of which produces a single vagus response volley as determined by the cathode ray oscillograph, a long lasting shock of sufficient intensity will, in certain preparations, stop the heart, *i. e.,* stimulate *n* vagus fibers. If the shock duration be made shorter, or the intensity be made less, so that each shock stimulates only *n/a* fibers, *at least a* shocks must be delivered to the nerve before the heart is stopped. Since the effect of a single vagus volley does not last indefinitely, the condition of repeated stimulation may require that more than *a* shocks be used, this number being dependent upon the frequency of stimulation. It seems probable that a similar argument can be applied to the other so-called iterative nerves.

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[2] Lapicque and Meyerson, *C. R. de la Soc. Biol.*, Paris, 1912, **72**, 63.

5581

Serum Sickness in Rabbits.*

MOYER S. FLEISHER AND LLOYD JONES.

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

It is well known that serum sickness appears in a very large proportion of individuals injected for the first time with normal horse serum or with various antisera. For the experimental study of this reaction it is of value to be able to reproduce in laboratory animals a condition analogous to serum sickness in man. We believe that the results reported below are the first ones concerning the occurrence of serum sickness in rabbits.

When a single injection of horse serum is given to rabbits in sufficient quantities either intravenously, intramuscularly, subcutaneously or into the subscapular tissues, there appears, from 3 to 7 days after the injection, a reaction characterized by erythema and edema. This reaction is evident on the rabbit's ears; there is noted a generalized flush which may involve the entire lower three-quarters of the ear, and which may be diffuse, confluent and of even intensity, or diffusely distributed, mottled and with discrete darker patches; the edema appears characteristically in the basal third of the ear but may extend upward to involve the entire lower two-thirds of the ear. The edema is frequently so marked that the pitting subsequent to pressure can be noted visually. The erythema and edema may each occur alone but both are usually present. These reactions can easily be differentiated from those occurring in the normal rabbit's ears; here hemorrhages, petechial or more extensive, and small circumscribed patches of erythema may be noted. Such appearances are very common. At times edematous areas often associated with trauma or hemorrhage occur; in the normal animal these latter reactions are usually noted in the upper half or two-thirds of the ear, and such areas were found in only 35 of 147 normal animals which were observed for periods of 2 to 8 weeks.

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* We wish to express our appreciation of the kindness of both Eli Lilly & Company and the H. K. Mulford Company in supplying us with both normal horse serum and the immune sera used.

In a total of 98 animals receiving an adequate amount of normal horse serum, positive reactions (generalized erythema, a basal edema or both) were observed in 66; the percentage of positive reactions being therefore slightly less than 66%. In a majority of the animals the reactions appeared subsequent to injection of the serum into tissues far removed from the rabbits' ears. It appears that at least 5 cc. of serum per kilogram must be injected into the rabbit to bring about a positive result. Quantities as high as 10 cc. per kilogram have been effective. In 8 animals injected with 3 cc. per kilogram no reactions were noted. During the experiments 5 different sera were used. The reactions most frequently appeared on the fifth and sixth days after injection. White rabbits are more satisfactory for use in such experiments, but the reactions have been observed in either brown or black rabbits.

In a more limited number of animals receiving various antisera, similar reactions were produced.

In a number of animals receiving a second injection of horse serum from 4 to 6 weeks after the first one either immediate or accelerated reactions appeared in which edema and erythema were manifest within several hours to 3 days after the injection

Studies of the precipitin and precipitinogen in the blood of the injected animals at various periods after injection have not demonstrated any obvious relationship between the time of appearance of the precipitin or the observed titers of antigen and antibody on the one hand, and the occurrence of the reactions of serum sickness in the rabbit on the other hand.

5582

Virulence and Electrophoresis of Bacillus Diphtheriae.

LLOYD JONES. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

Thirty-two cultures of *B. diphtheriae* isolated from as many diagnostic throat cultures and 2 laboratory stock cultures (No. 300 and Park No. 8) all of which exhibited characteristic morphology and fermentative reactions with carbohydrates, were examined for virulence and for velocity of electrophoretic migration.

All of the cultures fermented dextrose and galactose. Some of the cultures utilized dextrin when first isolated while other strains acquired the ability to utilize this substance after continued laboratory cultivation. None of the strains fermented sucrose. Accord-

TABLE I.

Culture No.	P. D. μ/sec./volt.	Virulence (Guinea Pig)
32	.260	X
13	.340	—
1	.377	X
30	.387	X
20	.394	X
15	.394	—
14b	.395	—
9	.395	X
300	.412	X
Park-8	.416	X
21	.418	X
19	.420	X
37	.440	X
28	.457	—
10	.480	X
39	.490	X
7	.502	X
14	.505	—
31	.512	X
22	.537	X
34	.537	X
3	.550	X
6	.557	—
11	.592	X
13a	.595	X
12	.640	X
17	.650	X
35	.677	X
38	.690	X
16	.692	X
5	.714	X
29	.735	X
26	.737	—
14a	.750	X

ingly, upon the basis of carbohydrate fermentation all cultures were considered typical of *B. diphtheriae.*

Virulence was determined by intracutaneous inoculation of guinea pigs. Electrophoretic mobilities were determined by the use of a Falk micro-electrophoresis apparatus and velocities of migration calculated as micra per second per volt.

The data indicate that virulence or lack of virulence of these organisms for the guinea pig is not correlated with the observed velocity of electrophoretic migration, since 27 virulent cultures exhibited widely divergent mobility rates. P. D. varying from 0.260 to 0.750 and similarly in 7 avirulent cultures P. D. varied from 0.340 to 0.737 μ/sec./volt.

From several virulent cultures, aberrant avirulent types were developed through forced dissociation and in these latter lack of virulence was not correlated with any significant change in the rate of electrophoretic migration.

Agglutinability of cultures with high and with low P. D. in the presence of salts, such as calcium chloride and copper acetate, was determined. In general, cultures having low P. D. as indicated by low velocity of electrophoretic migration, were agglutinated by a lesser concentration of electrolyte than were the cultures having higher P. D. values. However, as noted in the electrophoresis observations agglutinability in salt solution did not bear significant relationship to the virulence of the culture

5583

Further Experiments on Artificial Immunity to a Larval Cestode.*

HARRY M. MILLER, JR. (Introduced by J. Bronfenbrenner.)

From the Department of Zoology, Washington University.

Certain aspects of the general problem of the immunity of the albino rat to *Cysticercus fasciolaris,* begun in 1929,[1] have been investigated. In one set of experiments rats were given series of immunizing injections of: (a) a 1% suspension of powdered worm material *(Taenia taeniaeformis,* the adult stage of *C. fasciolaris)* in physiological saline solution; (b) an 0.84% suspension of powdered worms which had been subjected to prolonged extraction with ether in a Soxhlet or Pickel extraction apparatus; (c) a 1% suspension of finely ground worms which had been quickly frozen while alive with carbon dioxide snow and stored in evacuated glass ampoules for 3 months at —10°C.; (d) a 1% suspension of powdered worm material of *Taenia pisiformis*, a closely related species from the intestine of the dog. All rats, with litter mate controls, were fed with equal portions of a uniform suspension of onchospheres. At autopsy 35 days later normal sized cysts (4-5 mm. diameter) were present in the livers of control animals; while development of the larvae had been completely, or almost completely inhibited in the rats of groups a, b, and c. In these the larvae were represented for the most part by minute spots. In the livers of those animals injected with powdered *T. pisiformis* material (group d) the cysts developed as well as in the control rats, so that it has been demonstrated that no non-specific immunity developed from the injection of this closely-related worm material.

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* This investigation, and others in progress, was made possible by a generous grant from the Rockefeller Foundation.

[1] Miller, H. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 926; *ibid.*, 1931, **28**, 467.

5584

Effect of Theelin upon the Developing Ovary of the Rat.

E. A. DOISY, J. CURTIS AND W. D. COLLIER.

From the Departments of Biochemistry and Pathology, St. Louis University School of Medicine.

This report is the first of a series based upon the studies of the effect of theelin, a female sex hormone isolated by one of us from the urine of pregnant human females, upon the genital tract of the female white rat. This report is confined to the histological study of the effect of subcutaneous injections of solutions of this substance upon the ovaries of immature female rats.

A group of 14 controls, usually taken from the same litters as the experimental animals, was killed at different age periods to establish normal developmental changes. One group of 11 animals was injected daily with 3 rat units of theelin for varying periods of time, beginning all animals on the twenty-first day and killing each animal on the last day of theelin administration. A second group of 9 animals was given a single massive dose of 20 rat units on the first day of the experiment, the twenty-first day of life, and then were allowed to recover for varying periods of time.

The animals were killed by ether anesthesia. The genital tracts were immediately removed and fixed in 10% formalin. The ovaries were later dissected away, imbedded in paraffin and serial sections prepared. The size of the various ovaries was determined by preparing camera lucida drawings of each serial section, measuring the outlines by the polar planometer, adding these measurements to obtain the total area, reducing this figure to absolute size and multiplying by the thickness of the section. There is often so great a variation in the size of the ovary on the 2 sides and in the size of ovaries of animals of the same age that a graphic chart, plotting age against size, has been prepared. (Not given in this report.)

The growth curve of the normal animals is almost a straight line throughout the fourth and fifth week of life corresponding to the first and second week of the experiment. This growth is due to 3 obvious factors; greater development and increase in size of many of the follicles, increase in the amount of loose fibrous stroma in the medullary portion and increased vascularity. The theelin continuous administration series maintained the same growth rate and presented the same histological appearance as did the normals during the first week of the experiment. The recovery after a single massive dose series shows a complete inhibition of growth, a relatively greater atretic degeneration of follicles and moderate hydropic degeneration of germinal epithelium during the first week.

The ovaries of the normal animals almost double in size during the second and third weeks of the experiment. The theelin continuous administration series shows only one-sixth as great a growth as the normals, while the recovery from a massive dose series shows recovery from previous inhibition and a greater rate of growth than the normals. The absolute size of the recovery series is still about 10 days behind that of the normal series and, histologically, the previously engendered hydropic degeneration of the germinal epithelium is not entirely recovered from, although there is a progressive increase in size and number of developing follicles and progressive return to the normal hyperchromatic, small germinal epithelial cell type. Histologically, the series receiving theelin daily shows progressive degeneration of follicles, progressive inhibition of the development of new follicles and progressive hydropic degeneration of the germinal epithelium.

During the fourth week of the experiment, the normals developed mature follicles, ovulated and produced the first crop of corpora lutea. This phenomenon was accompanied by a sharp increase in size, almost doubling the size in a single week. The continuous administration series showed only about one-sixteenth the normal increase in size for this period, and, showed only a few well formed follicles and an increase of the intensity of the hydropic state of the germinal epithelium. The recovery series shows a growth rate of comparably normal rate although absolute size remains 10 days retarded. The histological picture is normal.

During the fifth and sixth weeks of the experiment when the animals were 8 and 9 weeks old, the normals showed only a very slight increase in size due to the fact that the ovary has about completed its growth. The continuous administration series showed even less increase in size. The recovery series which had previously recovered from the histological effects of a single massive dose of theelin and has since showed only 10 days' retardation in growth, ovulated 10 days late and appears identical in size and appearance with a normal of the same age.

The effect of the continuous administration of 3 rat units of theelin for a period of 5 weeks was to retard growth almost completely after the first week of the experiment, to decrease the number of developing follicles, to increase the amount of atretic degeneration of follicles and to produce a most remarkable hydropic metamorphosis of the germinal epithelium. A few follicles will reach maturity, rupture, and become luteinized apparently, since 2 small corpora were seen in one and one in another of the 2 animals carried longest.

5585

Immunity Studies Among Central and Polar Eskimos.*

J. RALPH WELLS AND PETER HEINBECKER.
(Introduced by J. Bronfenbrenner.)

From the Washington University School of Medicine, St. Louis.

This report is based upon material and data collected within the Arctic Circle by one of us (P. H.) during the summer of 1930 for the purpose of checking the work which led Heinbecker and Irvine-

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* This investigation was made possible through the cooperation and generosity of the Department of the Interior, Northwest Territories and Yukon Division, Ottawa, Canada; the National Research Council in Medicine; and Washington University. The laboratory phases of this study were carried out in the Department of Bacteriology and Immunology, Washington University School of Medicine, by the senior author.

Jones[1] to state "That the immunity shown does not depend on infection by the diphtheria bacillus," and that "it emphasizes the probability that the antitoxic mechanism is not as specific as is ordinarily supposed."

Cultures and direct smears from throats of 115 different individuals, 19 specimens of sera, and the results of 60 Schick tests comprise the basis of this report, representing 7 isolated groups of Central and Polar Eskimos of all age-groups and involving practically the entire population of the 7 localities visited. In these cultures and smears, organisms morphologically resembling *Corynebacterium diphtheriae* were recovered from 48 of the 115 individuals examined.

On the basis of fermentation reactions, the strains here reported represented a number of different groups, corresponding rather closely to those given by Andrewes[2] and his coworkers. Judging by results of these tests, which were made about 3 months after the beginning of the study, no true *Corynebacterium diphtheriae* were found. However one strain (Bache 4) resembled true diphtheria bacilli in that it fermented dextrose and dextrin but not sucrose and mannitol at the time it was isolated. This resemblance was further evidenced by the fact that 1 cc. of a 48-hour broth culture killed a normal guinea pig weighing 205 gm. within 10 days, whereas the same quantity of the culture did not affect a normal guinea pig of 185 gm. weight which had been given 350 units of diphtheria antitoxin 2 hours previously. These results were again obtained with the same broth culture 3 months later.

Schick tests were made on 2 groups of Eskimos, using material secured from the United States Public Health Service. Results are given in Table I.

TABLE I.

Group	No. tested	Children and young adults			Adults		
		No.	%+	%—	No.	%+	%—
Pond Inlet	39	14	64	36	25	52	48
Pangnurtung	21	6	33	67	15	27	73

Fifty-five per cent of the group of young individuals and 44% of adults gave positive Schick reactions, a total of 47% of all the persons examined, as compared to 25% of 49 such tests reported in 1928.[1]

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[1] Heinbecker and Irvine-Jones, *J. Immun.*, 1928, **15**, 395.

[2] Andrewes, *et al.*, *Med. Res. Council*, London, 1923, 44.

Nineteen specimens of serum, representing 4 groups of Eskimos, were titrated for diphtheria antitoxin content, using the method of Flood.[3] The results, in units per cubic centimeter, are shown in Table II.

TABLE II.

Group	No. tested	No. showing antitoxin None	1/45	1/22	>1/22
Pond Inlet	8	5		1	2
Dundas Harbor	2			1	1
Robertson Bay	6	3	1	1	1
Bache	3	2	1		
Total	19	10	2	3	4

More than half of the sera examined possessed no diphtheria antitoxin.

Because of these data and the fact that the respiratory flora in general was found[4] to be very similar to that of groups living elsewhere, we are inclined to believe that the mechanism of the production of immunity to diphtheria among Central and Polar Eskimos is much the same as it is among persons living in other latitudes.

Such a conclusion is strengthened by Bay-Smith's report[5] of outbreaks of clinical diphtheria among Greenland Eskimos, as well as the results of his 684 Schick tests showing 59% positive reactions. This investigator, although admitting that clinical diphtheria has been known to exist at least in certain groups of Eskimos, believes in a "spontaneous" origin of diphtheria antitoxin in the Schick negatives of the 684 Kap-Farvel and Julianehaab Eskimos. An outbreak described as clinical diphtheria in Central Eskimos has also been reported by Boas.[6]

5586

The Blood Sugar of Normal Fasting Persons.

B. Y. GLASSBERG. (Introduced by Michael Somogyi.)

From the Laboratory of the Jewish Hospital of St. Louis.

It has long been known that blood contains non-sugar substances that, in alkaline solution, reduce copper and other metals used in sugar determinations. These reducing non-sugars are non-fermentable and account for the "residual reduction" of blood after yeast fermentation.[1] The blood sugar values recorded generally in the literature all represent the sum of the sugar and the non-sugar reducing substances. Somogyi[2] devised a technique of fermentation of blood with washed yeast for the accurate determination of residual reduction, thus making it possible to determine the true sugar by deducting the residual reduction from the total reduction value. Benedict[3] and Folin[4] also devised useful methods for the determination of true sugar. Somogyi[5] perfected a very simple method of precipitation with zinc salts whereby together with the blood proteins the reducing non-sugars also are precipitated and thus filtrates are obtained that contain no appreciable amount of reducing substances other than sugar. Using such zinc filtrates, true sugar values are obtained in a single determination by any of the titrimetric or colorimetric methods in general use. Somogyi[6] has obtained virtually identical results in zinc filtrates by the Schaffer-Hartman-Somogyi, the Folin-Wu, the modified Folin-Wu, and the Benedict procedures. Filtrates prepared by the older methods of deproteinization, on the other hand, yield sugar values that are too high and in addition variable according to the method employed, due to the differences in reducing power of the non-sugars with various reagents. This situation gave rise to much confusion and made impossible the correlation of results from different investigators. The possibility of the determination of true sugar makes it imperative that the confusing and misleading variety of "apparent" blood sugar values be discarded and true sugar values determined in their stead.

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[3] Flood, *Am. J. Dis. Child.*, 1930, **39**, 107.

[4] Being reported elsewhere.

[5] Bay-Smith, *Klin. Woch.*, 1929, **8**, 974.

[6] Boas, The Central Eskimo, *Sixth Annual Rep. Bur. of Ethnology*, 1884-1885.

As a step to this end I have determined the fasting true sugar in the blood of 100 healthy normal adults, 50 men and 50 women. The blood samples were taken before breakfast, at least 8 hours after the last meal. Somogyi's microtechnique for zinc precipitation combined with the Shaffer-Hartmann method[7] was employed throughout this work. Blood was obtained by puncture of the finger tip and collected either directly into specially calibrated capillary pipettes or more conveniently, 5 to 6 drops were allowed to fall into a small beaker containing a minute amount of finely powdered potassium oxalate. In this work each result represents the average of 3 parallel determinations. In order to obtain a sufficient amount of blood, the hand was previously warmed in a basin of warm water.

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[1] Otto, J. J., *Arch. d. Ges. Physiol.*, 1885, **35**, 467.

[2] Somogyi, M., *J. Biol. Chem.*, 1927, **75**, 33.

[3] Benedict, R., *J. Biol. Chem.*, 1928, **76**, 457.

[4] Folin, O., and Malmros, H., *J. Biol. Chem.*, 1929, **83**, 121.

[5] Somogyi, M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 353.

[6] Somogyi, M., *J. Biol. Chem.*, 1929, **83**, 157.

[7] Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

The question may be raised whether capillary blood may be substituted for venous blood in sugar determinations. Our results corroborate Foster's[8] findings that in the fasting state the sugar content of capillary and venous blood is virtually the same. The degree of accuracy of the microtechnique was examined in the following experiment: In 17 cases sufficient blood was obtained from the finger to make parallel determinations by the micro and macro methods. In the latter the usual 1:10 dilution was employed. The results show that the values obtained by the 2 methods differ only by amounts that are within the limits of experimental error permissible for micro methods. All determinations were carried out at least in duplicate, usually in triplicate, with good agreement except in 2 cases. Comparing the results obtained by the macro and micro methods it is observed that the differences range between —4 and +8 mg. per 100 cc. of blood. The average of the discrepancies is 2.6 mg., the micro method yielding slightly lower results.

TABLE I.

Showing sugar content of successive blood samples taken from the same puncture.

Case No.	Mg. of sugar per 100 cc. of blood.				
	In specimens number			Greatest difference	Difference between first and last specimens
	I	II	III		
32	65	76	72	11	7
33	74	76	78	4	4
34	80	87	82	7	2
38	83	87	78	9	—5
42	72	80	79	8	7
43	76	87	84	11	8
44	85	73	72	13	—13
45	83	82	80	3	—3
48	87	84	86	3	—1
49	95	95	95	0	0
50	76	72	72	4	—4
51	79	83	88	9	9
52	76	84	84	8	8
53	85	90	92	5	7
57	88	76	73	15	—15
59	90	95	91	5	1
60	82	82	81	1	—1
62	87	90	84	6	—3
63	81	74	74	7	—7
64	72	80	87	15	15
Average				8.5	0.9

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[8] Foster, G. L., *J. Biol. Chem.*, 1923, **55**, 291.

For most purposes the sugar values obtained by the micro technique are sufficiently accurate.

Changes in the composition of the blood due to admixture of tissue juices, consequent to repeated squeezing of the finger, have been assumed to affect the sugar content of finger blood. To obtain evidence on this point, successive blood samples were collected from the same puncture and the sugar determined separately in the several samples. Table I shows the results of 20 such experiments. These figures prove that any differences in the sugar content of these successive samples are within the limit of experimental error and not due to the admixture of tissue juices. It is found that the average difference between the first and last sample is only 0.9 mg. %.

Based on the foregoing findings it is fair to assume that the figures given in Table II represent actual fasting true blood sugar values of the persons analyzed. (The only selectivity employed in obtaining these persons was to reject any women in the catamenia.) The lowest blood sugar obtained was 70, the highest 95, the average of all cases was 81.6 mg. per 100 cc. of blood.* It is noteworthy that in the majority (63%) of the cases, the sugar values are within the rather narrow limits of 75 to 85 mg. %, and only 9 cases show more than 90 and 9 cases less than 75 mg. % of true blood sugar.

TABLE II.

Showing the fasting true blood sugar of fifty male and fifty female normal adults.

Blood sugar, mg. per 100 cc. blood	Number of cases
Above 95 mg. %	0
90-95 ”	9
85-89 ”	19
80-84 ”	35
75-79 ”	28
70-74 ”	9
Below 70 ”	0
Total number of cases	100
Maximum blood sugar	95 mg. %
Minimum ” ”	70 ”
Average ” ”	81.6 ”

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* Since this paper was prepared for publication, the communication of G. W. Holt and E. M. Greisheimer entitled “ ‘True’ glucose tolerance in forty-two normal individuals” appeared (PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 547). These authors found the true fasting sugar in 42 normal persons to be 83 mg. %, a figure in close agreement with the average of 81.6 mg. % reported in this paper. These results extend the statistical value of the analyses here given.

Summary. 1. The fasting blood sugar of 50 men and 50 women was found to be between 70 and 95 mg. per 100 cc. of blood, with an average of 81.6 mg. %. 2. Capillary blood may properly be substituted for venous blood in determining the sugar of fasting individuals. Admixture of other tissue fluids does not modify the sugar content of finger blood.

Minnesota Section.

University of Minnesota, May 20, 1931.

5587

Glycogen Formation from Amino Acids.

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From the Department of Physiology, University of Minnesota.

One lot of control rats was kept on stock diet until killed in order to establish the glycogen and lipid contents of the liver without a starvation period. A second lot of control rats was starved for 24 hours before killing, from which group the fasting level was derived.

The remaining rats were starved 18 hours, then fed the amino acid to be investigated, and killed 6 hours later. Forced feeding was resorted to, except in the case of tyrosine. This amino acid, suspended in water, was applied to the rats' hair about the head and neck region. The animals cleaned it off nicely, thereby getting practically all of it into the gastro-intestinal tract. The other amino acids used did not lend themselves readily to this method of feeding, in which studies, feeding was performed by means of a medicine dropper. The disadvantage of the latter method lies in the rather large amount of water which must be introduced in keeping the amino acid conveniently suspended. The amount of amino acid fed in each case was approximately 2 gm.

The amino acids were purchased from the Eastman Kodak Company. They were d-l alanine, l-cystine, d-glutamic acid (practical), glycine, and 1-tyrosine.

The results are presented in Table I. The mean body weight, liver weight, liver weight expressed as % of body weight, % of liver glycogen, and % of liver lipid are tabulated. In each case the results for the males are shown first, those for the females second, and those for the sexes combined, finally. The number of each sex studied is indicated in the first column. Lipid determinations were not made in every case.

Owing to the small number of cases, the statistical method of analysis proved impractical. The group fed alanine contained several young animals, which accounts for the lower body weight, and

TABLE I.

Procedure and Number	Mean body weight (gm.)			Mean liver weight (gm.)			Liver as % of body weight			Mean liver glycogen (%)			Mean liver lipid (%)		
	Males	Females	Combined	Males	Females	Combined	M.	F.	C.	M.	F.	C.	M.	F.	C.
Unstarved 4 M.—4 F.	269	236	252	7.508	6.490	6.992	2.81	2.74	2.78	4.11	3.35	3.73	3.20	3.83	3.52
Starved 4 M.—4 F.	337	233	285	7.932	5.619	6.776	2.35	2.40	2.37	0.37	0.20	0.28	5.24	—	—
Alanine 7 M.—10 F.	250	152	192	6.919	5.092	5.845	3.17	3.56	3.40	1.02	1.61	1.37	6.45	5.03	5.69
Cystine 3 M.	443	—	—	10.307	—	—	2.34	—	—	0.77	—	—	5.49	—	—
Glutamic Acid 2 M.—5 F.	420	263	308	8.593	5.617	6.468	2.08	2.25	2.20	0.53	0.22	0.31	6.37	8.82	8.12
Glycine 4 M.—4 F.	285	215	250	6.895	5.112	6.003	2.85	2.37	2.61	0.13	0.53	0.33	5.26	5.61	5.43
Tyrosine 3 M.—5 F.	351	251	289	7.593	5.994	6.593	2.15	2.37	2.29	0.75	0.79	0.77	5.10	—	—

for the higher value of the liver expressed as per cent of body weight. The lowest glycogen content was found in the fasting group, but the values for the glutamic acid- and glycine-fed animals are so slightly above the fasting level, that we may consider no glycogen to be formed from either of these acids in the present method of investigation.

Glycogen was readily formed from alanine, as expected, and the feeding of cystine was likewise followed by definite glyconeogenesis.

The surprising result was the evident formation of glycogen from tyrosine. This amino acid is treated as ketogenic, in diabetic diet calculations, but under the conditions of our experiments, it appears to be antiketogenic.

The total lipid content of the liver is low in the unstarved rats, quite high in those fed glutamic acid, and between 5 and 6% in the other groups.

5588

Correction for Undercooling in Material of High Solid Content.

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As a solution is cooled in a freezing-point determination, crystallizing does not start at the freezing temperature. The temperature falls below the freezing-point, to an extent varying in different cases, before the freezing process commences. As soon as ice does start to form, the temperature climbs rapidly toward the freezing-point. The heat for this rise of temperature is derived from the freezing process, the latent heat of fusion of ice being 80 calories. The formation of ice is equivalent to the removal of water, and hence the solution will be concentrated. The freezing-point observed will, therefore, represent a solution more concentrated than the original solution; or, to express the fact differently, the freezing-point depression observed will be greater than the true depression for the solution.

The solution, if the amount of solid present is so small that it can be neglected, will be concentrated s u/1 times for u degrees of undercooling (Jones[1]), where s equals the specific heat of the solution and 1 the latent heat of fusion. Thus the true freezing-point, Δ, will equal the observed freezing-point, Δ^1, minus $\frac{s\,u\,\Delta^1}{1}$.

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[1] Jones, H. C., *Z. f. Phys. Chem.*, 1893, **12**, 624.

$$\Delta = \Delta^1 - \frac{s\,u\,\Delta^1}{1} \quad (1).^2$$

Assuming the specific heat to be unity and 1 to be 80,

$$\Delta = \Delta^1 - \frac{u\,\Delta^1}{80} \quad (2).^*$$

However, if there is a large percentage of solid present in the material, then, for the same amount of undercooling, there will be a greater concentration in such material than in a solution containing only a negligible amount of solid. The same quantity of ice must be formed in each case (assuming the specific heats to be the same), and hence the concentration will be greater in the material of high solid content. If the solids are considered, the correction equation becomes

$$\Delta = \Delta^1 - \frac{s\,u\,\Delta^1}{80\,w} \quad (3).$$

The term, s, is the specific heat of the material, and w represents the fraction by weight of water acting as a solvent (more exactly, this water plus the soluble constituents dissolved in it).

It is instructive to obtain undercooling data for materials of widely varying water contents. Solutions of NaCl and KCl have been taken as examples of solutions of low solid content. The observed freezing-point, Δ^1, was measured for different degrees of undercooling, and the results were recorded on coordinate paper, Δ^1 being plotted against undercooling. It is possible to obtain the true freezing-point, Δ, from such a graph; for Δ corresponds to Δ^1 at zero undercooling, and can hence be obtained from the graph by extrapolation.† By substituting in equation (3) the value obtained for Δ and also a value of Δ^1 and its corresponding u (obtained from the graph), w can be calculated. With the NaCl and KCl solutions, w ranged around unity. This result is to be expected since the solid content of a 1% NaCl or KCl solution is negligible from the present viewpoint.

In the same manner, w has been determined for whole blood, serum, and corpuscles. However, the values obtained did not correspond to the actual total water contents of such material. For ex-

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[2] Harris, J. A., and Gortner, R. A., *Am. J. Bot.*, 1914, **1**, 75.

* The amount of heat required to raise the temperature of the mercury and glass of the thermometer and of the glass of the freezing-point vessel is not considered in this correction. However, the specific heat of mercury is only 0.033 at 0°C., and that of glass is approximately 0.2.

† This is practically a straight line relationship.

ample, in an experiment on the corpuscles of beef blood, a value of 0.34 for w was obtained. The fraction of water in beef corpuscles is about 0.60. The discrepancy may possibly be explained by the presence of water not active as a solvent ("bound water") at the temperature of the freezing-point. Accordingly, the bound water content of the beef corpuscles in the above experiment is 26%.

Thus the equation, $\Delta = \Delta^1 - \frac{s\,u\,\Delta^1}{80\,w}$, may be used in correcting for undercooling in the determination of the freezing-point in solutions of high solid content, the w in the equation for a given material being determined from freezing-point data on undercooling. Where large amounts of solid are present, it is important to make this correction. The undercooling data also provide a method for determining bound water in a rough manner; that is, the fraction of total water present minus the w of the above equation equals the fraction of the bound water.

NOTE: Dr. F. H. MacDougall has kindly developed an equation in which the specific heats of the various components are taken into account separately.

$$\Delta = \Delta^1 - \left[1 + \frac{(w_2 + w_3\,c)}{w_1}\right]\frac{u}{80}\Delta^1.$$

In this equation w_1 equals the fraction of actual solution, w_2 the fraction of bound water, w_3 the fraction of solid material, and c the specific heat of the solids. The specific heats of the solution and of the bound water are assumed to be equal to unity.

5589

Action of Ultra-Violet Rays on New and Old Bacterial Cultures.

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Cultures of bacteria placed on agar are as a rule easily killed by exposure to ultra-violet light from a quartz mercury arc. The amount of radiation necessary for devitalization depends upon several factors. Perhaps the most intricate of these is the variation of resistance with age. This relation between resistance and age has not been determined in detail. The following study was aimed as a contribution to our knowledge in this respect.

In the following experiments a new Victor quartz mercury arc lamp was used without any filter. The current was 4.5 amperes at 65 volts and the distance from burner to plate was 24 inches.

An old culture was one that was grown and transplanted daily for one week and then sealed and left in the museum till needed; a period of from one to 6 weeks.

A new culture was one that was grown and transplanted daily from the old culture for a period of 5 weeks or so. *Coli communis* was used as the culture in most of our experiments, a control being run on each plate irradiated.

Series I. A small amount of culture was removed with a wire loop 2 mm. in diameter and this was mixed with 2 cc. water. Then one loopful of this suspension was spread on one-half of an agar plate and another loopful on the other half and these were marked "control" and "irradiated".

Series II. As much of the suspension as would adhere to the wire loop was removed from the agar after the exposure and planted in 5 cc. of beef broth. The same was done with the controls.

All cultures were irradiated for ¾ min. Four plates were used in each set of experiments. One irradiated immediately on transplanting from old or new culture, one each irradiated 1, 3 and 6 hours after transplanting with the results as shown on chart Series I and Series II.

In the above series where only 2 or 3 broth or agar plates showed growth at ¾ min. no growth was visible if a similar plate was irradiated for 1 min.

In Series I and II, 4 new and 4 old plates were irradiated together. The number which showed growth after exposure is shown in Column 3.

	Age of Culture	Number of Plates	
		Growth	No Growth
Series I	38 days	0	4
	1 "	4	0
	34 "	1	3
	1 "	4	0
	38 "	0	4
	1 "	3	1
	34 "	1	3
	1 "	3	1
Series II	38 "	1	3
	1 "	3	1
	34 "	2	2
	1 "	4	0
	38 "	3	1
	1 "	4	0
	34 "	1	3
	1 "	4	0

In the above two series 29 out of 32 new cultures resisted radiation whereas 9 out of 32 old cultures grew after the exposure.

In another series of experiments it was found that portions of old cultures which were grown for 1, 3, 6, 12, 18 and 24 hours and then irradiated were more resistant to ultra-violet than portions of the same cultures which were irradiated immediately after transplanting. Thus even after one hour some of the cultures showed still greater resistance. They gained resistance from the time of transplantation up to about 24 hours, but their resistance to ultra-violet was diminished with greater age as shown in Series I and II.

The "increased resistance" of the colonies might be due either to greater individual resistance or to the greater number of bacteria present some time after transplantation. We tried to differentiate between these factors by means of bacterial counts.

Consistant results of the counts were not obtained when the bacteria were planted on agar and therefore a peptone solution was used instead for these experiments.

One cc. peptone culture media with one wire loopful of *Coli communis* was diluted with 99 cc. sterile water to make a 1/100 solution. This was No. 1. Ten cc. of this No. 1 was diluted with 90 cc. water yielding a 1/1000 solution labeled No. 2. Ten cc. of No. 2 solution was added to 90 cc. water, giving a 1/10,000 dilution. This was solution No. 3. The diluting was continued in the same way until a 1/10,000,000 solution was obtained, *i. e.,* solution No. 6.

One-fourth cc. of each of the above solutions were spread on agar plates and irradiated. If more than ¼ cc. was used the solution would adhere to the sides of the plate by capillary attraction and the rays could not reach it. One cc. of each dilution was used and suspended in agar and plated for our control count.

We multiplied the number of colonies left after irradiation by 4 to get results based on one cc. of the suspended culture.

In Series V, VI, VII, VIII and IX the above was again repeated but with a greater difference in the ages of the various growths.

It is well known that older cultures have smaller numbers of bacteria than new cultures, and our experiments bring this out clearly. They also indicate, however, that the individual resistance of the bacteria to ultra-violet light is reduced. (This is interesting to note, as it is generally stated that old bacteria are more resistant to heat, boiling, antiseptics, etc.)

We found that the resisting powers toward the ultra-violet rays are quickly built up by reculturing, even as in some cases by being

		Counts of No. of Colonies		Calc. No. of Colonies per 1 cc.	
		Not Irr. (per 1 cc.)	Irr. (per ¼ cc.)	Not Irr.	Irr.
Series V	1 Day Old				
	Dilution I	—	100	10,500,000	400
	Dilution II	—	25	1,050,000	100
	Dilution III	—	6	105,000	24
	Dilution IV	—	0	10,500	0
	Dilution V	—	4	1,050	16
	Dilution VI	105	0	105	0
Series VII	37 Days Old				
	Dilution I	—	3	53,000	12
	Dilution II	—	0	5,300	0
	Dilution III	—	—	530	—
	Dilution IV	53	—	53	—
	Dilution V	20	—	5.3	—
	Dilution VI	2	—	0.53	—
Series VIII	60 Days Old				
	Dilution I	—	4	1,050,000	16
	Dilution II	—	4	105,000	16
	Dilution III	—	0	10,500	0
	Dilution IV	—	0	1,050	0
	Dilution V	105	0	105	0
	Dilution VI	17	1	10.5	4
Series IX	80 Days Old				
	Dilution I	—	0	3,000	0
	Dilution II	—	0	300	0
	Dilution III	—	0	30	0
	Dilution IV	3	—	3	—
	Dilution V	1	—	.3	—
	Dilution VI	1	—	.03	—

grown on new media for only one hour. Cultures which have been grown for about 24 hours have the greatest resistance to ultra-violet ray. When they are transplanted their resistance drops down and increases again with time so that after 12 hours it has come up to almost the same resistance as that of a 24-hour culture.

In connection with the above experiments, some other studies were made of which we wish to mention the results.

A suspension of *Oidium albicans* in Saboraud Agar was plated and exposed to 2 minutes of ultra-violet light at 12 inches. It was found that enough rays to kill the bacteria penetrated through the agar whose thickness averaged 0.66 mm. according to micrometer measurements. Bacteria below 0.66 mm. showed definite growth.

Staphylococcus, *Oidium albicans, Proteus vulgaris* and *Aspergillus fumigatus* were also used in some experiments which were carried out as Series I but consistent results of the killing time were not obtained in these preliminary tests.

Suspensions of *Oidium albicans* in agar were irradiated with X-rays, a coverglass was the only filter used (190 kv. at 60 cm. from the tube, 30 milliamperes) for 3 hours. The growth seemed retarded but on transplanting it grew normally. *Staphylococcus aureus* and *Coli communis* were also irradiated with the X-rays for varying lengths of time up to 5 hours but no change was noted in the growth of the bacteria.

We are very much indebted to Dr. Arthur T. Henrici for his help in supplying us with the cultures and for giving us advice and suggestions.

5590

Comparative Study of the Quantity of Gas in the Bowel in Simple and Closed-Loop Obstruction.*

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The results of previous studies have indicated that whereas the accumulation of gas as detected by X-ray examination is a reliable agent in the early diagnosis of simple obstruction, in strangulation obstructions Roentgen evidence is not of the same value in its early recognition.[1, 2, 3] The results of the following study are based on direct measurements of gas in simple and closed-loop obstructions.

Method: Studies of 36 dogs form the basis of this report. Dogs with closed-loop rather than strangulation obstructions were used in this comparison because the dogs with strangulation obstructions only survive for short periods. In the closed-loop, as a rule there are no gross vascular changes until late in the course of the obstruction at which time closed-loop obstruction partakes of the features of strangulation obstruction. In the closed loop obstruction the oral source of gas has been eliminated and in establishing this type of obstruction every trace of gas was "milked out" of the bowel before the last bowel end was inverted. Thirty-one closed-loop obstructions of small bowel of varying lengths and at various sites were established by the usual standard methods. The dogs were then observed for various lengths of time and either killed when they appeared moribund or were reoperated upon before they reached this stage. At operation the closed-loops were removed and im-

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* This work was supported by Grant 188 allowed by the Committee on Scientific Research of the American Medical Association.

[1] PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 674.

[2] PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 952.

[3] PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 343.

mediately thereafter the gas and contents were measured by aspiration with a syringe and hollow needle. The length of gut, site and duration of obstruction were accurately recorded in all cases. In some the diameter of the bowel was also measured. If the dogs were killed, an autopsy was performed immediately after death and similar measurements of the gas, fluid contents, etc., were made. Dogs that died at night in which immediate examination could not be carried out are excluded from this series as are all dogs that showed evidence of leakage peritonitis or any other lethal complication at time of operation or killing. Measurements of gas in the proximal bowel of 5 dogs with simple obstruction were made. These dogs were killed and the pylorus clamped. The fluid and gas intervening between the pylorus and the obstruction were measured.

Data showing the type of obstruction, average duration in hours or days, the site and average length of gut obstructed and the average amount of gas found are here tabulated.

TABLE I.
Closed-loop Obstruction.

No. of dogs	Site	Av. length gut (cm.)	Av. No. hrs.	Av. No. cc. gas	Av. No. cc. fluid
20	Lower ileum	57.8	82.1	15.0	91
11	Upper jejunum	32.0	31.0	8.5	40

Simple Obstruction.

Experiment	Obstruction	Time in days	Gas in cc.	Fluid in cc.
1	Lower ileum	5	350	350
2	,, ,,	3	80	80
3	,, ,,	4	160	180
4	,, ,,	14	525	590
5	,, ,,	9	525	900

The relatively small amount of gas in the closed-loops bespeaks the importance of the oral source for the greater quantity of intestinal gas in bowel obstruction. McIver[4, 5] and his associates have previously emphasized swallowed air as the significant factor in postoperative distension. The quantity of gas accumulating in the bowel under obstructive conditions (closed-loop) through the agency of bacterial activity does not appear to be great. The smallest amounts observed in simple obstruction are greater than any found in the 31 closed-loop obstructions.

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[4] *Arch. Surg.*, 1926, **13**, 588.

[5] *Am. J. Physiol.*, 1926, **76**, 92.

In the first 3 dogs with simple obstruction only the last 2 feet of small bowel proximal to the obstruction were found distended at all. In dogs 4 and 5 the last 3 or 4 feet were distended. These latter 2 dogs more nearly approximate the average distension usually seen at autopsy in this type of obstruction.

In 7 of the closed-loop animals, no gas at all could be found in the loops. The greatest amount found was 75 cc. and the second largest amount was 35 cc. In this last dog, 600 cc. of fluid material was also recovered from the loop. As to variations in lengths of the loops the shortest was 12 cm. and the longest 100 cm. These lengths compare favorably with the lengths of gut found to be dilated in dogs having simple obstruction. As to a comparison of the time factors in the 2 series, 6 of the dogs with closed-loops of the ileum lived more than 4 days before they were sacrificed. One lived 7 days and one 10 days. One dog not included in this series because of death occurring during the night lived 29 days with a 3 foot loop of the terminal ileum. The time factor in the 2 series is practically the same.

In another study, strangulation obstructions have been established in 36 dogs. This type of obstruction was brought about by tying off the mesenteric blood supply with umbilical tape and then wrapping the tape around the outside of the bowel wall at the proximal and distal ends of obstructed segment. In this manner the lumen of the bowel was obstructed as well as its blood supply. The oral source of gas was thus eliminated in these obstructions. Although no direct measurements of gas in the first 30 dogs of this series were made, frequent attempts to aspirate gas with a needle and syringe at the operating table failed to reveal any gas in these loops. In 6 of the dogs in which the loops were resected, no gas could be found when the usual technique for gas collection was employed. The short duration of these experiments lasting from 3 to 12 hours probably accounts for the fact that gas from bacterial sources is of no practical consequence.

Summary: In simple obstructions gas accumulates in the proximal bowel in far greater amounts than within closed-loop or strangulated segments. Gas formation in the latter 2 types is an inconstant and irregular feature. Gas accumulation in these instances is due largely to the activity of intestinal bacteria. The exclusion of swallowed air from the closed and strangulated loops accounts largely for these significant differences.

5591

On the Nutritive Value of Certain Oils.

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Following the work of Burr and Burr,[1, 2] a study of several oils has been made and it has been noticed that cod liver oil affects the animals in a way different from the common edible oils. This has been pointed out recently by Sinclair[3] and by Graham and Griffiths.[4] These workers find that the feeding of cod liver oil does not prevent scaliness of the feet and tail and our findings are in agreement with this view. Although in our earlier work, we thought the animals receiving cod liver oil were always normal in appearance, in later work we have found a considerable number of animals which were receiving 2 to 5 drops of cod liver oil daily with scaly feet and tails.

However, cod liver oil does cause renewed growth. Table I shows the increases in weight of sick animals after the feeding of cod liver oil.

TABLE I.

Date	Number in Group	Cod Liver Oil	Average wt. increase in 5 weeks	Condition of skin after feeding dose
1928	3 males	2 drops	68 gm.	Scaly
1928	6 females	2 ”	37 ”	”
1930	3 ”	5 ”	22 ”	”

The 3 females in the 1930 group all mated and produced litters. This is another indication of much improvement over the fat-free animals.

Of the more unsaturated oils, cod liver oil is distinctive, in that it leaves the skin of many of the animals scaly. Lard, olive oil, corn oil, linseed oil, methyl linolate and methyl linolenate all produce skin free from scales and dandruff.

It should be pointed out, however, that cod liver oil is highly unsaturated without linolic and linolenic acids being present in ap-

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[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

[2] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

[3] Sinclair, R. G., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1059.

[4] Graham, C. E., and Griffith, W. H., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 756.

preciable quantities.[5] Although Suzuki and Masuda[6] list both linolic and linolenic as being present this is disputed by Denisov[7] and others. Our feeding tests indicate that they certainly are not present in appreciable quantities. In this respect it is different from all other oils studied in this work. As shown by Sinclair[3] cod liver oil in the diet markedly increases the degree of unsaturation of the phospholipids of the animals consuming it. This is further evidence that the structure of the acids is more important than the degree of unsaturation. The possibility of a toxic effect of the cod liver oil, suggested by Graham and Griffith[4] must of course be considered.

Sinclair[3] has observed that his animals do not become scaly but do remain subnormal in weight if they have access to their feces. We have been unable to completely confirm this. As a preliminary experiment 2 females (31004 and 31005) were kept in individual cages on shavings. After 16 weeks on a "fat-free" diet their feet were only slightly scaly, but there was a trace of dandruff and the tails were moderately scaly with swollen, red tips. They were not in as bad condition as their sisters on wire bottoms, but they were not normal. There was undoubtedly some beneficial effect of the feces or the shavings. Their weights were 158 g and 168 g, respectively.

At the same time that the above animals were started 4 cures were attempted, 2 with the fat extracted from the feces of animals on the "fat-free" diet 550-B and 2 with 20% of powdered feces added to the diet. The results of these experiments are given in Table 2.

TABLE II.

Rat Number	Dose Fed Daily	Weight Increase in 5 weeks	Condition of Skin after 5 weeks
		gm.	
30161	5 drops fecal fat	—8	Much scale
30137	5 ″ ″ ″	+7	″ ″
30132	20% dry feces	+3	″ ″
30151	20% ″ ″	—8	″ ″

From our experience it seems that the effects of feces are small, leaving the animals at a subnormal weight and probably exerting a slight prophylactic effect over a long period of time, but not giving effective cures in a period as short as 5 weeks.

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[5] Bills, C. E., *Chem. Rev.*, 1926, **3**, 425.

[6] Suzuki, B., and Masuda, Y., *Proc. Imp. Acad.* (Japan), 1928, **4**, 165.

[7] Denisov, I., *Chem. Abst.*, 1930, **24**, 6047.

Conclusion. From the data at hand it would seem that the highly unsaturated fatty acids of cod liver oil can be used by fat deficient rats for growth, but that their scaly skin is cured only by linolic and linolenic acids, which, apparently are lacking in cod liver oil.

It may prove possible to isolate a fat which will cure the skin without producing growth, as indicated by Sinclair's experience.

The fat deficiency may therefore resolve itself into 2 factors. It so happens that linolic and linolenic acids seem to relieve both growth and skin abnormalities. The relative values of several fatty acids in the prevention of the above symptoms as well as kidney degeneration is being included in our present studies.

5592

Individual Variation in Serum Calcium in Normal Men and Women.*

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From the Students' Health Service and Department of Preventive Medicine and Public Health and the Department of Physiology, University of Minnesota.

Since the work of Bell[1] many investigators have studied the blood calcium level in relation to menstruation. The results of these investigations have been contradictory. Watchorn[2], Close and Osman[3], Allen and Goldthorpe[4], Spiegler[5], and others have concluded that there is very little or no change in the blood calcium in relation to the menstrual cycle. Sharlit et al[6] and Matters[7] report a premenstrual rise in blood calcium and a menstrual fall in the calcium level. Okey et al[8] state that "while the changes in the concentration of serum calcium at any phase of the monthly cycle are not outstanding, there is some tendency to frequency of low values for calcium a few days previous to the onset of menstruation and to frequency of higher values from the 8th to the 15th days following the onset of menstrual bleeding."

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* This study was carried out with the aid of a grant from the Research Fund of the University of Minnesota.

[1] Bell, Blair W., *Proc. Royal Soc. Med.*, 1908, **1**, 291.

[2] Watchorn, Elsie, *Brit. J. Exp. Path.*, 1926, **7**, 120.

[3] Close, H. G., and Osman, A. A., *Biochem. J.*, 1928, **22**, 1544.

[4] Allen, E., and Goldthorpe, H. C., *Am. J. Obs. and Gyn.*, 1929, **17**, 789.

[5] Spiegler, R., *Arch. Gyn.*, 1930, **143**, 201.

[6] Sharlit, H., Corseaden, J. A., and Lyle, W. G., *Arch. Int. Med.*, 1927, **39**, 780.

[7] Matters, R. F., *Australian J. Exp. Biol. and Med. Sc.*, Part 2, 1929, **6**, 119.

[8] Okey, R., Stewart, J. M., and Greenwood, M. L., *J. Biol. Chem.*, 1930, **87**, 91.

With the exception of the work of Okey et al[8], these findings have been based upon either the study of very small groups of individuals or upon blood calcium determinations taken not more than 3 or 4 times during a menstrual cycle.

Experimental work done on lower animals shows that the blood calcium level is definitely affected by the reproductive system. Riddle and Reinhart[9] found a tremendous increase in calcium in the blood of birds prior to ovulation. Mirvish and Bosman[10] have produced a decrease of 2 to 3 mg. in the plasma calcium in both rabbits and human beings by injecting ovarian follicular and corpus luteum extracts.

The present study was undertaken in an effort to determine standards of blood calcium in relation to the menstrual cycle in women with normal menstrual periods in order that we might have some basis for comparison in a study being made on a group of women with dysmenorrhea. As a control the blood calcium levels and variations in a group of men were studied.

Serum calcium determinations were made in duplicate on 14 women and 11 men 3 times a week for 4 consecutive weeks. Determinations were made on 3 of the 14 women for a period of 8 weeks, giving a total of 17 weekly readings on women. In a few instances, more than 3 determinations were made in one week and in an occasional instance only 2 were made. In each case, the blood was drawn between 8:30 and 9:30 A.M. The subjects were all healthy individuals. No attempt was made to determine the type of diet the individual was eating. The determinations were all made during the months of November and December, 1930, and January, February, and March, 1931.

The determinations of serum calcium were made by one of us (E. M. G.) using the Clark-Collip[11] modification of the Kramer-Tisdall[12] method. A total of 132 readings were made on the group of men and 157 on the group of women.

Tables I and II present the range in the amount of serum calcium and the mean of the 12 serum calcium determinations for each individual in the group of men and women respectively.

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[9] Riddle, O., and Reinhart, W. H., *Am. J. Physiol.*, 1926, **76**, 660.

[10] Mirvish, L., and Bosman, L. P., *Quar. J. Exp. Physiol.*, 1927, **18**, 11, 19.

[11] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[12] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **47**, 475.

TABLE I.
Serum Calcium Variations in a Group of 11 Men.
Tri-weekly determinations for 4 weeks. Of 12 determinations.

Case No.	Age	Low	High	Mean	P. E.	S. D.
1	36	9.69	11.01	10.39	±0.0703	0.361
2	28	10.08	11.67	10.95	±0.0547	0.281
3	28	9.80	10.54	10.15	±0.0355	0.1824
4	31	9.77	11.23	10.29	±0.0773	0.3968
5	32	9.89	10.38	10.14	±0.0327	0.1679
6	38	9.85	10.68	10.24	±0.0434	0.2137
7	25	9.99	10.90	10.45	±0.0527	0.2592
8	27	10.32	11.05	10.69	±0.0592	0.3039
9	43	9.75	10.31	10.00	±0.0332	0.1634
10	39	9.85	10.66	10.17	±0.0661	0.3392
11	24	10.03	10.74	10.24	±0.0461	0.2051
Means	31.91	9.91	10.83	10.31		0.2612
		Range .92				±0.0532

TABLE II.
Serum Calcium Variations in a Group of 17 Women.
Tri-weekly determinations for 4 weeks. Of 12 determinations.

Case No.	Age	Low	High	Mean	P. E.	S. D.
1	40	9.34	10.43	9.91	±0.0838	0.3929
2	22	9.68	10.43	10.10	±0.0520	0.2441
3	25	9.53	11.14	10.08	±0.0868	0.4261
4	27	9.30	10.25	9.70	±0.0655	0.3072
5	24	9.28	10.39	9.75	±0.0794	0.3532
6	23	9.39	10.35	9.91	±0.0582	0.2860
7	28	9.52	10.31	9.93	±0.0466	0.2289
8	30	9.54	10.31	9.90	±0.0463	0.2170
9	29	9.74	10.55	10.15	±0.0568	0.2664
10	35	9.44	10.35	9.88	±0.0607	0.2701
*11 (7)	28	9.29	10.74	10.12	±0.1093	0.4570
*12 (3)	25	9.35	11.25	10.53	±0.1167	0.5193
*13(10)	35	9.41	10.62	10.01	±0.0948	0.3712
14	24	9.00	10.86	10.00	±0.1292	0.5405
15	23	9.80	11.19	10.31	±0.0893	0.3974
16	34	9.57	10.59	10.03	±0.0936	0.3914
17	21	9.37	10.33	9.96	±0.0882	0.3220
Means	27.82	9.44	10.59	10.01		0.3523
		Range 1.15				±0.0576

*Duplicate cases.

The mean age of the men was 31.91 years and that of the women was 27.82 years.

The mean serum calcium for the entire group of men was 10.31 mg. and for the group of women 10.01 mg. The average calcium level for the women was 0.30±0.0282 mg. lower than the level for the men, a difference that is of definite significance. Although it is generally stated that there is little variation in the blood calcium level between the sexes, in these groups the men showed a consistently higher level of serum calcium than the women.

The variability or range of calcium level is distinctly greater for the women than for the men, the mean range for the women being 1.15 mg. and for the men 0.92 mg. Nine of the 17 women show a range of more than 1 mg. while only 3 of the 11 men show such a range. This greater variation in calcium levels in women was also observed by Okey.[8]

TABLE III.
Calcium Level in Relation to Menstrual Cycle.

Week	Mean mg. Calcium	P. E.	S. D.	Difference of Means
Rest	10.05	±0.0419	0.3349	0.07±0.0640
Pre-Menstrual	10.12	±0.0484	0.4485	0.19±0.0616
Menstrual	9.93	±0.0388	0.3905	0.04±0.0591
Post-Menstrual	9.97	±0.0447	0.4090	

In order to compare the calcium levels at various phases in the menstrual cycle, the cycle was divided into 4 weekly periods hereafter designated as the rest period, the pre-menstrual period, the menstrual period and the post-menstrual period. The menstrual period included the calcium determinations made on the first day of the period and during the following week. Because of the daily variation in calcium levels, the mean readings for each week of these menstrual phases were computed as it was believed they would give better data for comparison.

Table III presents the mean serum calcium level for each period in the menstrual cycle. The highest calcium level is 10.12±0.0484 mg. in the pre-menstrual period and the lowest of 9.93±0.0388 mg. in the menstrual week. The difference of 0.19±0.0616 mg. is probably significant. It is to be noted also that the mean calcium levels for each week of the menstrual cycle, if plotted, would form a fairly regular curve.

TABLE IV.
Mean Serum Calcium Values for Men in Chronological Order.

	Mean Calcium	P. E.	S. D.	Difference of Means
First Week	10.16	0.0301	0.2961	0.13±0.0481
Second Week	10.29	0.0380	0.3471	0.01±0.0509
Third Week	10.30	0.0368	0.3140	0.25±0.0648
Fourth Week	10.55	0.0532	0.4322	0.39±0.060

Table IV presents the mean serum calcium for each week of the 4 weeks chronologically for men. The variation in serum calcium from week to week is greater than that for women arranged according to the menstrual cycle. With the men there is found to be a definite upward trend from week to week. This was not found in women when the weekly mean calcium values were computed on a chronological basis disregarding the menstrual cycle. However, the fact that with the men there is this definite increase from week to week and the fact that it is recognized that there is a seasonal variation in blood calcium make us consider the possibility that normal chronological variations may affect the values given in Table III although in the data we have, this effect is not evident.

Since the lowest weekly mean of serum calcium in women occurred during the menstrual week and since there was no comparable biological week in the men, it was decided to tabulate the weekly

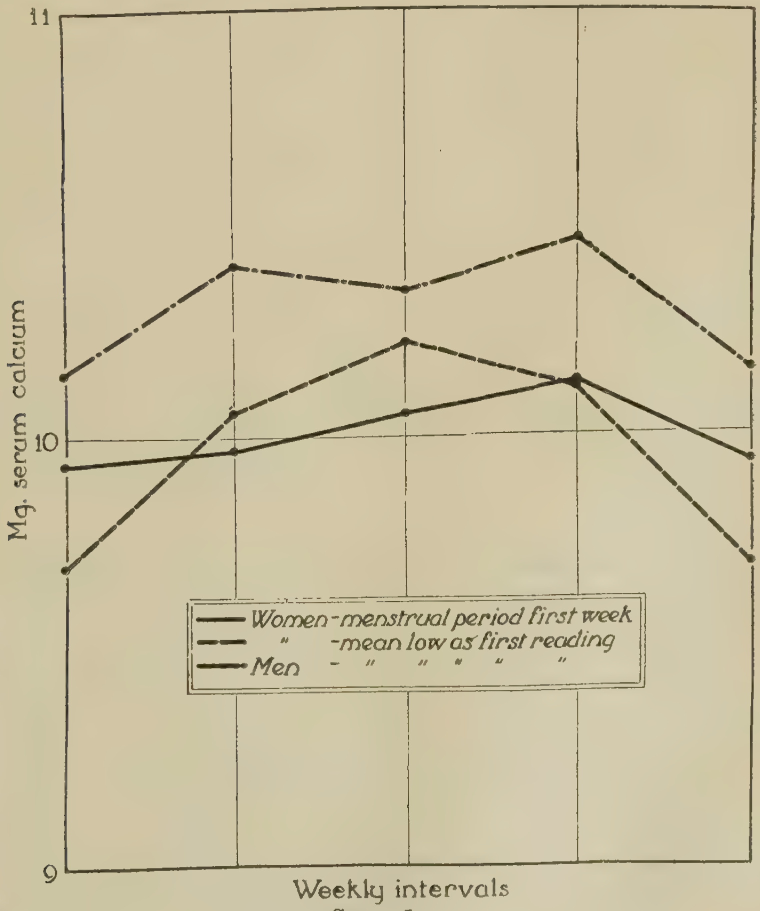

CHART 1.

mean calcium values for each individual in the 2 groups, putting the lowest mean weekly value as the first reading and the following 3 weekly mean values in sequence. We felt this method would give a better method for comparison of the men and women. These data are presented in Tables V and VI.

This treatment of the data further substantiates the finding previously given that the serum calcium level in men is higher than in women, since in each, the mean value for men is higher than that for women.

Table VI shows that in 12 of the 17 individual weekly mean calcium readings, the lowest value occurs in the menstrual or post-menstrual week. In 5 of the 17 cases the highest value occurs in the pre-menstrual week.

The mean serum calcium values for the entire group of men (Table V) starting with the low week shows an irregular variation from week to week. With the women (Table VI) it is seen that the weekly variation assumes the same type of regular curve as when the values for women were grouped according to the menstrual week. This is shown graphically in Chart I.

From this evidence it seems probable that women show a cyclic variation in blood calcium while men do not. It is appreciated, however, that it will be necessary to carry a group of women through more than one menstrual cycle before this supposition can be proved.

Summary. From a determination of serum calcium on 17 women and 11 men at tri-weekly periods for 4 weeks, the following is found:

1. The serum calcium level for women is definitely lower than

TABLE V.
Serum Calcium Variations in Men. Comparison of Weekly Averages.

Case No.	Low	Second	Third	Fourth
1	10.21	10.24	10.79	10.33
2	10.51	10.99	10.79	11.20
3	10.07	10.25	10.15	10.14
4	9.94	10.28	10.16	10.78
5	9.94	10.22	10.35	10.07
6	10.12	10.28	10.20	10.31
7	10.27	10.66	10.28	10.59
8	10.53	10.85	10.57	10.82
9	9.90	9.95	10.10	10.08
10	10.01	10.47	10.11	10.08
11	10.07	10.20	10.23	10.55
Means	10.14	10.40	10.34	10.45

TABLE VI.
Serum Calcium Variations in Women. Comparison of Weekly Averages.

Case No.	Low	Second	Third	Fourth
1	9.50*	9.61	10.29	10.28
2	10.00	10.29	10.04*	10.10
3	9.60	10.03	10.43*	9.97
4	9.32	9.78*	9.88	9.89
5	9.49*	9.66	9.61	10.37
6	9.51*	10.03	10.17	9.97
7	9.75	10.15	9.94	9.80*
8	9.81*	9.84	10.05	9.98
9	9.95	10.17	10.50	10.10*
10	9.72	9.80	10.02	10.06*
11	9.84	10.04	10.99	10.11*
12	9.97	10.28	10.96	10.68
13	9.74	10.62	10.19	9.80*
14	9.55	9.79*	10.47	10.56
15	10.04*	10.75	10.50	10.12
16	9.76*	10.35	9.92	10.09
17	9.37	9.99	10.24*	9.94
Means	9.70	10.07	10.22	10.11

*Menstrual week.

for men. 2. There is a greater daily variation in the serum calcium for women than for men as well as a greater absolute range in calcium levels. 3. There is a tendency for the serum calcium to be highest in the pre-menstrual period and lowest in the menstrual period. This is evident in the mean serum calcium values for each week in the menstrual cycle. The fact that in 12 of 17 individuals, the lowest weekly mean serum calcium occurred in the menstrual or post-menstrual period further substantiates the finding that there is a fall in blood calcium following menstruation. 4. In women there seems to be a regular cyclic curve of blood calcium level which is not present in men.

5593

Variations in Plasma Phosphatase, P., Ca., and Glucose Following Administration of Glucose and Calcium Glycerophosphate.

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From the Department of Pediatrics, University of Minnesota.

In the course of studies on the calcium and phosphorus metabolism of children suffering from various disorders, it was observed that the level of plasma phosphatase tended to be lower than normal in patients with pseudo-hypertrophic progressive muscular dystrophy and in those with poliomyelitis with extensive paralyses. Kay[1] has reported a marked increase in the concentration of this enzyme in cases of *Osteitis deformans* and in certain other diseases of bone. These variations suggested to us the desirability of determining whether or not the level of phosphatase in the blood can be altered by any simple means. We have, therefore, determined the phosphatase and at the same time the inorganic phosphorus, calcium and glucose of the blood after the administration of water, of glucose and of calcium glycerophosphate in children with minor ailments or with various chronic disorders.

Following 400 cc. of drinking water alone no significant alterations in the blood values were found. The usual changes in blood sugar and phosphorus resulted from administration of 50 gm. of glucose in 400 cc. of water. A slight rise in the level of serum calcium was observed at the end of 6 hours. Although there were transient variations in the phosphatase in all of the cases receiving glucose, the only significant alterations were found in one case of progressive muscular dystrophy and in one of extensive muscular paralysis from poliomyelitis. In both of these the initial values, which were subnormal, had nearly doubled at the end of 6 hours.

The total number of cases in which complete studies of the effect of glucose, of calcium glycerophosphate or of water alone were made was 15 children.

The most striking results obtained were those following the administration of 20 gm. of calcium glycerophosphate in 400 cc. of water. While the inorganic phosphorus remained unchanged, the phosphatase, calcium and glucose each showed a slight temporary rise followed by a profound fall at the end of 6 hours. In 2 essentially normal subjects the blood glucose fell to 41 and 44 and the serum calcium to 6.0 and 6.8 mgm. per 100 cc., respectively, without the ordinary clinical signs of hypoglycemia or of tetany. Further study of these reactions must be made before their real significance can be evaluated.

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[1] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 235.

5594

Action of Peptides in the Nutrition of Young Mice.

J. F. MC CLENDON.

From the Laboratory of Physiological Chemistry, University of Minnesota.

It seems impossible to make young mice grow at a normal rate on any mixtures of known amino acids substituted for the protein of the diet, no matter what the proportion of the amino acids. By adding the vitamin B in the form of yeast slight growth is obtained, but by adding the vitamin B fraction in the form of the Osborne-Wakeman preparation there is usually a decline in weight. Since the active principle in the cure of pernicious anemia as well as insulin, secretin, and glutathione, are all said to be peptides, it seems probable that peptides are necessary in nutrition. The end products of both gastric and pancreatic digestion of proteins contain peptides, and it seems probable that they, to some extent, would be absorbed as such. If the stomach is removed from a mammal, death finally occurs accompanied by anemia. A dog may live for some months or a man for some years without a stomach but finally dies. A person with pernicious anemia has no gastric digestion and when fed the gastric digest of a protein is cured of the symptoms. It may take a long time to find just what peptides are necessary. So far the results are: Young mice fed on a mixture of the known amino acids, some radicals of prosthetic groups vitamines, mineral salts, carbohydrate, and fat, decline in weight and health. Control mice fed on this diet to which has been added all of the peptides which I have yet been able to obtain, decline at a less rapid rate, are more lively and apparently healthy, and live longer than those not receiving the peptides.

If casein is hydrolyzed with acid, there is a progressive hydrolysis of all imid linkages, accompanied by a slow destruction of the amino acids. Some peptides do not give the biuret reaction and I know of no way of proving the total absence of peptides except by the purification of the amino acids in the hydrolysate. The hydrolysis may be continued until mice no longer grow when it is substituted for protein in the diet. There is no growth on casein hydrolyzed for 36 hours with boiling acid, even though all of the known amino acids are added to it to make up for any that might be destroyed. It seems possible that casein hydrolyzed for 16 hours or less, may contain minute traces of peptides or diketopiperazines.

Since the experiments have continued 2 years and have been for the most part negative, only an example will be given in detail. Diet 15 contained in decigrams: glycine 2, alanine 4, valine 8, amino-n-valerianic acid 1, amino-n-butyric acid 1, aminoisobutyric acid 1, amino-n-caproic acid 1, leucine 10, isoleucine 1, proline 4, hydroxyproline 1, aspartic acid 10, glutamic acid 10, serine 1, phenylalanine 3, tyrosine 5, arginine 5, lysine 4, histidine 2, tryptophan 1, cystine 1, creatine 1, uracil 1, alloxan 1, spermidine 1, spermine 1, taurine 1, glucosamine 1, cholesterol 1, cellulose 1, glucose 75, dextrin 150, salts 6, Osborne-Wakeman vitamin B 15, butter fat 60. Diet 16 contained the same as diet 15 plus glycine anhydride 2, glycyl glycine 2, glutathyone 2, Dakin's liver extract, dipeptide of hydroxyproline and hydroxy-glutamic acid (impure) 20. Two white mice weighing 7.6 and 8.6 gm. were placed on diet 15 and 2 litter mates weighing 7.7 and 8.2 gm. were placed on diet 16. Their combined weights each day were:

Diet	Combined Weights Each Day.								
15	16.2	14.2	14.2	13.5	12.9	12.5	10	7.7	dead (not weighed)
16	15.9	15.0	14.8	14.0	13.9	13.1	12.3	12.0	12.0 lively

Since mice lived for months on diets similar to 16 and the experiments are so expensive, this experiment was discontinued when the controls died. The other experiments were similar except for variation in the proportions of the amino acids.

Southern Section.

Tulane University, May 21, 1931.

5595

Suppression of Strychnine Convulsions by Barbiturates.

W. T. DAWSON AND CHARLES H. TAFT, JR. (Introduced by M. Bodansky.)

From the Department of Pharmacology, University of Texas Medical School.

Zerfas and McCallum[1] have found amytal (ethylisomyl barbituric acid) an extremely effective and life-saving antagonist to strychnine in man and the rabbit. Our observations on unanesthetized rabbits show that this property is also possessed by barbital (diethyl barbituric acid), phenobarbital (ethyl-phenyl), nembutal (ethyl-secondary amyl) and pernocton (B-bromallyl secondary butyl).

In a first series of redbrown rabbits, of 1.05 to 1.87 kg., we induced tetanic convulsions by injecting subcutaneously 0.6 mg. of Merck's neutral strychnine sulphate per kg., using 1:1000 solution. Two controls died in 18 and 13 minutes respectively. All the others, 11 in number, returned to a normal reflex state following treatment with Na phenobarbital or Na barbital, given by ear vein immediately on onset of convulsions and repeated as judged necessary to control their recurrence. The minimal total amounts per kg. necessary to secure this result were found to be, Na barbital 40 mg., and Na phenobarbital 1 to 5 mg., indicating a much greater activity of phenobarbital. All of the "treated" rabbits were apparently normal next day, and were later used for other purposes.

In a second series of short haired albino rabbits of 1.24 to 2.03 kg., 0.6 mg. strychnine sulphate per kg., similarly given, was followed by spontaneous recovery in 3 animals, but 0.9 mg. per kg. killed 3 other animals in 27, 30 and 55 minutes respectively. In the antagonism experiments 1.2 mg. per kg. was used. The results were similar to those in the first series except that doses of the hypnotic barbiturates closer to the "general anesthetic" level were necessary. The suppression of convulsions required in individual animals total doses in mg. per kg. as follows: Na nembutal 4, 14, Na amytal 15, Na phenobarbital, 30, (32 days), 60, pernocton 40 (25 days), 40 (16 days), Na barbital, 150; for comparison, chloral hydrate, 220, of which 30 subcutaneously (31 days). The periods given indicate a survival to time of report; the other animals died in 2 to 11 days, possibly due to incomplete asepsis.

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[1] Zerfas, L. G., and McCallum, J. T. C., *Curr. Res. Anesth. and Analg.*, 1929, **8**, 349.

5596

Use of the New Born Mouse in the Study of Kidney Function.

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From the Department of Pharmacology, University of Texas School of Medicine.

A search of the literature has not revealed any satisfactory method of studying, by direct observation, the passage of dye through the mammalian kidney. The principal difficulties seem to be that the capacity and density of the kidney are so great that it has been impossible to see any appreciable distance into the kidney. The kidney of the new born white mouse is small and relatively transparent and appears to be a favorable subject for use in the study of kidney function. With proper illumination it is possible to see the various functional units in the living kidney.

The mice used in this work were from 2 to 24 hours old. They were injected subcutaneously, in the back, with a small amount (0.1 cc.) of some vital dye, such as 0.5% Indigo Carmine and immediately placed in a warmed cotton lined box. If the mouse is left undisturbed the dye appears in the urine in about 20 minutes.

Fifteen minutes after the injection the mouse is quickly etherized and a dissection made exposing one of the kidneys. The mouse is then placed in a special warm stage heated to 37°-38° C. and the kidney is kept moist with warmed Ringer's solution.

With a binocular dissecting microscope it is possible to distinguish if the injection is successful, the glomeruli and the presence of the dye along the tubules from the capsule to the pelvis.

In the same preparation it is possible to make a very pretty demonstration of peristalsis in the ureters. Clumps of dye can be seen passing down the lumen of the ureter propelled by its peristaltic movements.

5597

Infection Experiments in Monkeys with Human, Macaque and Ateles Strains of Strongyloides.

ERNEST CARROLL FAUST.

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Species of Strongyloides occur naturally in several primates. Those which have come under the observation of the writer have been in man, *Simia troglodytes, Pithecus rhesus, Ateles geoffroyi, Cebus capuchinus imitator,* and *Saimiri örstedi örstedi.* In man the infection occurs in 6.3% of the hospital and free-clinic patients in New Orleans and in 20% of the hospital and village population of Panama. Over a period of 2 years a colony of 12 rhesus monkeys in New Orleans has had an infection of 87.5%. Of 6 red spider monkeys from Panama 4 (66.6%) were positive, and of 3 capuchin monkeys and 15 marmosets one each was infected with Strongyloides. In man cultural studies made on material from cases observed by the writer showed both direct and indirect types with a preponderance of the direct type. All of the other strains studied have been consistently indirect in their mode of development.

Morphologically it is difficult to differentiate these primate strains, although they can be readily distinguished from the Strongyloides of the dog, the rat and the sheep. It has seemed desirable, therefore, to test certain of these strains by cross-infection experiments. For this purpose human, macaque and red spider strains, which had been studied over a period of several weeks, were selected. Six monkeys were inoculated by applying the inoculum to the abdominal skin for 30 minutes. Red spider monkeys (one each) were inoculated with about 10,000 f_2 larvae (1) of an autochthonous strain, (2) of a human direct strain and (3) of a *rhesus* strain. Rhesus monkeys (one each) were inoculated with about 10,000 f_2 larvae (1) of an autochthonous strain, (2) of a direct human strain and (3) of a red spider strain. A preliminary experiment had shown that the inoculum was equally viable, whether the larvae were washed free of the culture medium or not.

The red spider monkeys within an hour or two after inoculation showed evidence of an intense pruritus at the site of inoculation. This was followed for 2 or 3 days by a lupous dermatitis with moderate erythema. After 4 or 5 days the skin became normal again. On the second day after inoculation there was a severe croupous bronchitis without temperature, which was observed for a period of approximately 24 hours and then rapidly receded. In the animal inoculated with the autochthonous strain eggs were first recovered from the feces 18 days after inoculation. The animal lived 34 days after eggs were first recovered; at autopsy strongyloidiasis was determined to be the cause of death. In the case inoculated with the human strain the early symptoms were comparable to those of the autochthonous strain, but the incubation period was 22 days. Eggs in large numbers were recovered for 2 weeks, after which time the infection became greatly reduced and stools became consistently negative (direct examination and culture) from the 45th day after inoculation. *Hatched larvae* of this human strain were never obtained from freshly passed feces. The *rhesus* strain apparently never became implanted in the intestinal mucosa of the red spider monkey, since daily stool examinations from the time of inoculation for a period of 2 months were consistently negative.

The rhesus monkeys inoculated with the autochthonous and human strains showed a moderate pruritus but no gross dermatitis or erythema, and had no apparent bronchial involvement. However, in the case of inoculation of this host with the red spider strain of Strongyloides there was a profound erythema at the site of inoculation which was first noticed 24 hours after inoculation and became progressively more intense up to the fourth day, when lupous patches were also conspicuous. There were never any open lesions. After the seventh day the skin became rapidly normal again. On the third and fourth days after inoculation there was a slight bronchitis, which had disappeared on the fifth day. Intestinal infection in the rhesus monkey was apparently never effected with the red spider and human strains. Up to 2 months after inoculation the stools remained consistently negative. In the autochthonous strain eggs were first recovered on the twenty-fifth day after inoculation and from that time were found daily.

The evidence obtained from this series of experiments points to the conclusion that the *rhesus* and *red-spider* strains of Strongyloides are physiologically different species: they are easily inoculable into their natural hosts from which they were obtained, but they are unable to establish intestinal infections in the reciprocal hosts, although there is some evidence that they migrate through the tissues of the inappropriate host. The human strain can be established but is not able to maintain itself in the red spider monkey; in the rhesus monkey it appears to be completely abortive.

5598

Toxicity for White Rats of Elements Implanted into their Subcutaneous Tissues.*

JOHN W. WILLIAMS. (Introduced by C. W. Duval.)

From the Department of Pathology, College of Medicine, Tulane University.

With the view of observing the host response to various elements, the author has implanted such substances over the ribs of white rats anteriorly. The animals were anaesthetized with ether, a half inch incision made, the skin dissected up for a short distance, the element placed in position and the skin edges secured by skin clips. The whole procedure required less than 10 minutes and the clips were very effective. The following elements were used: aluminum, antimony, arsenic, barium, beryllium, bismuth, boron, bromine, cadmium, calcium, carbon, cerium, chromium, cobalt, columbium, copper, erbium, gold, iodine, iron, lead, lithium, manganese, magnesium, mercury, molybdenum, nickel, phosphorus, potassium, selenium, silicon, silver, sodium, sulphur, tantalum, tellurium, thallium, tungsten, uranium, vanadium, zinc and zirconium. Of the above elements only 2 proved fatal for the white rat—thallium and phosphorus, death occurring within 48 hours. This is in accord with the characteristics of the latter 2 elements, both thallium and the white phosphorus being poisonous. On autopsy, however, some of the phosphorus still glowed on being exposed to the air, showing that the absorption of the phosphorus was not rapid. The rats which survived were examined 7 and 30 days after implantation, and portions of tissue adjacent to the implantation were taken for histological examination. No marked reactions were noted around the implantations of aluminum, barium, beryllium, bismuth, boron, calcium, carbon, cerium, columbium, erbium, gold, iron, lead, magnesium, mercury, molybdenum, selenium, silicon, silver, tantalum, tellurium, tin, tungsten, uranium, vanadium, zinc and zirconium.

A large ulcer was noted in the case of titanium and a small ulcer in the case of bromine and potassium. Induration around the implantation was noted in the case of sulphur, antimony, arsenic, chromium, cadmium, sodium and lithium. Abscess was noted in the case of copper, nickel, manganese and cobalt and a greenish exudate in the case of iodine.

Approximately 75 rats were used and several implantations were

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* Aided by grant from Harrod Cancer Research Fund.

made in each. The weight of the elements was not taken since the author undertook this work from the standpoint of histological study of their effect on the tissues. The approximate size of the pieces of sodium, lithium, potassium, phosphorus and thallium placed at each site of implantation was 5 x 5 x 2 mm. and from one to 2 times this amount was used in the case of the other elements, with the exception of bromine, in which instance one cubic centimeter was placed in each area of implantation. The most striking feature of the work was the fact that a large number of the elements were practically inert when placed in the tissues. In this work the author hopes to correlate some of the physical properties of the elements with the tissue reactions and their histological study after a more lengthy series has been run. The reason why a lengthy series is necessary is because of the difficulty of sterilization in the case of some of the elements and also of keeping the animal wound sterile.

5599

Papain Preparations Suitable for the Prevention of Adhesions.

ROBERT P. WALTON. (Introduced by J. T. Halsey.)

From the Department of Pharmacology, School of Medicine, Tulane University.

The use of proteolytic enzymes in the surgical treatment of adhesions has indicated a definite superiority for the vegetable enzyme, papain.[1, 2, 3, 4] This report is concerned with the development of a sterile papain preparation suitable for clinical use, the standardization of the product and some descriptive aspects of its behavior in the peritoneum.

The two procedures previously described for the preparation of a sterile trypsin product[5] have been applied to papain with varying success. The simplest method, namely, the pressure filtration of a glycerine extract has been discarded as unsuitable for clinical purposes. Even though filtered glycerine extracts of trypsin lose very

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[1] Kubota, *Mitteilungen aus der Medizinischen Facultat der Kaiserlichen Kyushu-Universtat,* 1924, Bd.9, Heft 2.

[2] Ochsner and Mason, Proc. Soc. Exp. Biol. and Med., 1928, **25**, 524.

[3] Ochsner and Herrmann, *Arch. Surg.,* 1922, **17**, 365.

[4] Ochsner and Garside, yet unpublished.

[5] Walton, *J. Pharm. and Exp. Ther.,* 1930, **40**, 403.

little activity in the course of 8 months' storage and glycerine extracts of papain show a high degree of heat stability, the storage behavior of the latter is unfavorable. About 50 assays on 12 different lots over periods up to 4 months showed that filtrates stored open in the refrigerator averaged a 50% activity-loss in 1 month and ampouled filtrates stored in the refrigerator averaged a 50% activity-loss in 4 months. The cause for this deterioration was not determined although a number of possibilities were examined such as atmospheric oxidation, contact with mercury vapor, exposure to ultra-violet light and absorption of alkali from the container.

The second method, previously described, consisted in filtration of aqueous or glycerine extracts through Berkefeld filters (N) and precipitation of the active product with alcohol and ether. This presented no unexpected difficulty when applied to papain and the powdered products have shown consistent stability on storage. The firm of Parke Davis & Co. have cooperated in producing sterile and standard preparations by this method. The activity of the standardized, sterile product corresponds closely to that of the ordinary stock product, mgm. for mgm.

Papain preparations intended for oral administration have been assayed in drug houses by a method involving the digestion of raw meat. Further definitions of activity were considered desirable when standardizing preparations for this purpose. A method has been used based on formol titrations before and after digestion of purified gelatine. Another method, not as precise but simpler, is based on the time for disintegration of glycerine-stored fibrin. A third method used is a modification of the procedure described by Gates[6] and Gilman and Cowgill,[7] based on the extent of digestion of the gelatine on a strip of photographic film. In each of these methods citrate solutions have been used as "activator."

Because the effectiveness of papain was exhibited in such remarkably low activity-concentrations, Kubota[1] ascribed the effect to a chemotaxis with respect to leucocytes, leucoproteases presumably digesting the exudative fibrin. From our studies demonstrating the high degree of stability of papain it is suggested that the proteolytic activity of the papain itself might be adequate to explain its behavior. Aqueous solutions at 1-1,000 concentration were shown to retain about 55% of the original activity after standing 40 hours at 40°. This degree of stability was exhibited both by the purified sterile product and the ordinary stock product. Trypsin solutions (Fairchild's standard preparation), in contrast, lost 95% activity in 3 hours under the same conditions. Papain solutions were unaffected by the presence of serum in sufficient concentrations to inhibit trypsin almost completely at the same activity concentration. In the peritoneal cavity of dogs, papain solutions at concentrations of about 1-15,000 retained 50% activity after 5 hours.* Under similar conditions, the activity of trypsin disappears completely within 2 hours.[5]

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[6] Gates, *Science*, 1930, **72**, 147.

[7] Gilman and Cowgill, *J. Biol. Chem.*, 1930, **88**, 743.

The minimal fatal concentration for normal dogs may be placed at 1-2,000, when using 1 liter of isotonic saline per 10 kg. dog. At concentrations up to 1-300 there is marked irritation of the peritoneal surfaces and some hemorrhage. However, the amount of blood thus liberated, as indicated by hemoglobin determinations, has never exceeded 12 cc. and is thus not sufficient to explain the cause of death. Other possible explanations are being studied. Concentrations of 1-15,000 cause some irritation and occasionally slight bleeding, the amount of blood rarely exceeding 1 or 2 cc. These conclusions are based on experiments with 16 dogs. The extent of irritation caused by papain and by trypsin is about the same mgm. for mgm., as determined in 12 rats at concentrations of 1-2,000 and of 1-10,000. In this higher dilution there is little or no irritation with either. By way of reference it may be pointed out that papain has been considered effective in concentrations of 1-400,000.[1, 2, 4]

5600

Effect of Carbon Dioxide on Ethylene and Nitrous Oxide Anesthesia.

GEORGE B. KLEINDORFER. (Introduced by J. T. Halsey.)

From the Department of Pharmacology, Tulane University.

As a continuation of the investigation of the effects of CO_2 in anesthesia, this report embodies the results of a study of the effect of this gas on anesthesia by ethylene and by nitrous oxide. As shown in the accompanying graphs, it has been found that CO_2 exerts an effect on anesthesia by these gases of the same nature as on anesthesia with ether recently reported in this bulletin (Kleindorfer).[1]

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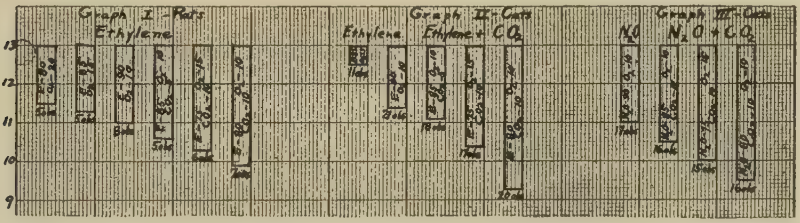
* Determined by activating removed samples with H_2S and assaying by the photographic film method. Addition of H_2S increases the activity about 36 times.

Thirty-six observations on the effects of mixtures of ethylene and oxygen with or without CO_2 were made on 10 white rats using the same methods employed in the ether series. The results are indicated in Graph I. However, the depth of the anesthesia procurable in these animals by these gases was so slight that it appeared desirable to supplement this series by observations on other animals. Because of the inconsistency of the reactions of rabbits to electrical stimulation of the divided sciatic nerve, which was the criterion used in this series, these animals were found not to be well suited to this type of investigation. While the results were in line with those in the rat series they are not reported in detail.



Change in strength of effective stimulus before and after administration of different gas mixtures as indicated by the change in distance of secondary from primary coils of inductorium.

The results of 87 similar observations on 12 cats are indicated in Graph II.

Results of 64 observations of the effect of CO_2 on nitrous oxide in 15 cats are similarly shown in Graph III.

It is apparent from the results reported that 5 or 10% of CO_2 exerts an unmistakable influence in anesthesia by ethylene or by nitrous oxide. These observations are in line with observations of Nicloux[2] and Henderson[3] and his coworkers. The former reported that he was able to obtain a good anesthesia from the inhalation of ethylene 65% oxygen 15% and CO_2 20%. The latter has reported an investigation with similar findings on the depth of anesthesia by substituting equivalent amounts of CO_2 for nitrous oxide. A detailed report of these observations will shortly appear elsewhere.

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[1] Kleindorfer, G. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 623.

[2] Nicloux, Maurice, *Soc. de Biologie*, 1925, **93**, 1650.

[3] Henderson, V. E., *et al.*, *Anesthesia and Analgesia*, 1930, **9**, 136.

5601

Effects of Triethanolamine on Hemolysis.*

JOHN W. WILLIAMS. (Introduced by C. W. Duval.)

From the Department of Pathology, College of Medicine, Tulane University.

The author's previous work[1] on the surface tension lowering substance, sodium taurocholate, and its effects on red blood cells as demonstrated by hemolysis suggested like observations on triethanolamine, N $(C_2H_4OH)_3$,[2] a new surface tension lowering compound now used commercially as an emulsifying agent and superior to similar agents previously used because of its low alkalinity, ability to cause finer and more stable dispersions, its slightly bactericidal powers, etc.

Approximately 25 human bloods were examined, a drop of blood drawn from the patient by syringe being added directly to each tube containing 2 cc. of the solution to be observed. Varying dilutions of sodium chloride, potassium chloride, calcium chloride, sodium hydroxide, hydrochloric acid, acetone, saponin, sodium taurocholate, venom from the bothrops atrox and sulphanilic acid were observed for hemolysis as such and containing 5% triethanolamine and the results compared. The influence of triethanolamine on the pH of the solutions and the time of hemolysis were also noted. The first readings registered were taken 2 hours after the addition of the blood. Normal sodium chloride was used as a vehicle with the exception of the sodium, potassium and calcium chloride dilutions and where otherwise noted. The pH of the distilled water and normal sodium chloride ranged from 5.6-5.8.

In the case of the sodium chloride dilutions, hemolysis began at the .40% concentration of the salt without triethanolamine and at the .30% concentration with the triethanolamine, the triethanolamine in each case definitely increasing the alkalinity of the solutions. Similar results were obtained with the potassium and calcium chlorides. Hemolysis began at .65% in the case of the potassium salt without triethanolamine and at .45% with triethanolamine, and the alkalinity of the dilutions was increased markedly by the tri-

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* Aided by grant from the David Trautman Schwartz Research Fund.

I wish to thank the Touro Infirmary for the blood specimens which made this work possible.

[1] Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 913.

[2] Wilson, A. L., *Ind. Eng. Chem.*, 1930, **22**, 143.

ethanolamine. Hemolysis began in the case of calcium chloride at .90% without and .60% with triethanolamine, and the alkalinity of the solutions was increased definitely by the triethanolamine.

In the case of sodium hydroxide a brown sediment appeared in concentrations of 1:128 and greater, both with and without the triethanolamine, and the pH was little affected. Likewise in the case of acetone a brown sediment appeared in concentrations of 1:4 and greater, both with and without the triethanolamine added. The pH of pure acetone changed from 5.4 to 6.8 on the addition of triethanolamine and the pH of the 1:2 dilution changed from 6.8 to 8.4 on the addition of the same substance.

In the case of hydrochloric acid hemolysis occurred in concentrations of 1:3360 and greater without triethanolamine, and in concentrations of 1:40 and greater with triethanolamine, the pH changing to 7.2 in the 1:20 dilution and to 8.4 in the 1:320 dilution on the addition of triethanolamine. Sulphanilic acid acted similar to hydrochloric. Hemolysis occurred in concentrations of 1:3200 and greater without triethanolamine, and in concentrations of 1:50 and greater with triethanolamine. The pH of the sulphanilic acid in the 1:50 dilution increased to 8.4 on addition of triethanolamine.

In the case of sodium taurocholate hemolysis began in concentrations of 1:100 without and 1:1600 with triethanolamine, and the triethanolamine in each case increased the alkalinity of the solutions. Saponin reacted in a somewhat similar but less pronounced manner, hemolysis beginning in the 1:6400 dilution without and the 1:12800 dilution with triethanolamine, and likewise the alkalinity of the solutions was increased by the triethanolamine.

In the case of venom, hemolysis did not occur either with or without triethanolamine in concentrations ranging up to 1:25 the latter of which was very opalescent. The alkalinity of the solutions, however, was increased.

Triethanolamine dilutions of 1:6389760 in distilled began to influence the pH toward the alkaline and at the dilution 1:24960 the pH was 8.4. Dilutions of triethanolamine in normal saline of 1:4 and greater do not as a rule show immediate hemolytic effects, nor do they show effects in 2 hours. However, there is a delayed hemolytic effect which occurs in 6 hours. A like delayed hemolytic effect was observed in all 5% triethanolamine dilutions of sodium chloride, potassium chloride, calcium chloride, sodium hydroxide, acetone, saponin, sodium taurocholate, venom with the exception of the 1:25 dilution which showed no hemolysis, sulphanilic acid with the exception of the unhemolysed dilutions of concentrations greater than 1:800 which took 32 hours, and hydrochloric acid with the exception of unhemolysed concentrations greater than 1:640 which took 18 hours.

In summary we will attempt to explain some of the differences which are apparent when triethanolamine is added. The temporary increase in resistance of red blood cells to hemolysis when triethanolamine is added to the sodium, potassium and calcium chloride dilutions might possibly be explained by the ability of triethanolamine to cause absorption of the salt on the surface of the red blood cells, or rather on the surface of the triethanolamine film surrounding the red cells, so that as a result the media adjacent to the red cells is less hypotonic than the figures would indicate, and equilibrium is maintained until the physico-chemical changes in the red cells destroy this equilibrium and hemolysis is effected. There is no or very little difference in the dilutions in which hemolysis takes place in the case of acetone and sodium hydroxide with and without triethanolamine, but it is to be expected that a substance of low alkalinity such as triethanolamine would have little effect on a strong alkali or a substance of the reactive properties of acetone. When triethanolamine is added to saponin and sodium taurocholate we in all probability have an addition of forces militating toward hemolysis, since we have substances toxic to red cells with abilities to lower surface tension and emulsify combined in one solution. In the case of hydrochloric and sulphanilic acids, triethanolamine probably acts as a protective of the red cell by forming a surrounding envelope and thus acting to maintain equilibrium between physico-chemical forces outside and those within the red cell. In addition, triethanolamine may interfere with the dissociation of these acids and thus reduce their activities. It should be noted here that the forces tending to maintain equilibrium are such that hemolysis is staved off longer in certain of the dilutions. In the case of venom very little effect is shown (unfortunately it was only possible to obtain a small quantity from the Antivenom Institute of America) and the probable reason why hemolysis did not take place in the 1:25 dilution when triethanolamine was added was that the phase was a triethanolamine and red cell in venom solution resulting in no triethanolamine being directly in contact with the red cell. Undoubtedly in the above case reaction plays a part, especially in the case of acids where dissociation is interfered with and the acid changed toward the alkaline. The maintenance of equilibrium where mentioned is an important factor in the delayed type of hemolysis. This delayed hemolysis to the author's knowledge has not been described for any substance or substances. This delayed hemolytic effect probably results from the gradual softening of the red cell resultant from the emulsifying action of triethanolamine on certain of its constituents with eventual complete dissolution of its structure.

5602

Oxygen Capacity and Hemoglobin Content of Normal Blood of Men.

PAUL C. FOSTER AND J. RAYMOND JOHNSON.
(Introduced by Henry Laurens.)

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana.

It is almost universally recognized that Haldane's figure for the oxygen capacity of human blood (18.5 vol. p. c.) is too low. In spite of this the old standard is still used. A recent text book[1] states that "the standard (referring to Haldane's) is regarded by American workers as too low," and Drinker,[2] in describing a hemoglobin standard, writes that "although the blood of normal men in this country . . . . contains on the average 16.6 gm. of hemoglobin which would correspond to an oxygen capacity of about 22 p. c., it was thought best to keep Haldane's standard for the present." It appears unreasonable to accept as a standard a scale on which every normal blood is well over 100 p.c. Haldane's figure was based on an original series of 12 men[3] and substantiated later by a second series of equal size.[4] Although Haldane's method of analysis has long since outlived its usefulness, and we now have an accurate and simple method for determining blood oxygen, no extensive observations on normal subjects have been reported.

We have determined, during the winter months, the oxygen capacity of the blood of 115 healthy male students ranging in age from 18 to 30, most of whom were born and have always lived in the South. Specimens were taken during the morning, oxalated (20 mg. per 10 cc.), and determinations made the same afternoon. The blood was oxygenated at room temperature with a gentle stream of air bubbles and the oxygen capacity determined on 2 cc. samples in the Van Slyke-Neill[5] constant volume manometric apparatus, the oxygen being determined directly by absorption with the sodium hydrosulphite reagent to which sodium anthroquinone-β-sulphonate was added as a catalyser. The determinations were checked within 0.2 cc. on two machines. The results are given in Fig. 1.

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[1] Wright, Samson, *Applied Physiology,* 1929, 3rd Ed., 205.

[2] Drinker, C. K., *Oxford Medicine,* **2**, 538.

[3] Haldane, J., and Smith, L., *J. Physiol.,* 1900, **25**, 331.

[4] Haldane, J., *J. Physiol.,* 1900, **26**, 497.

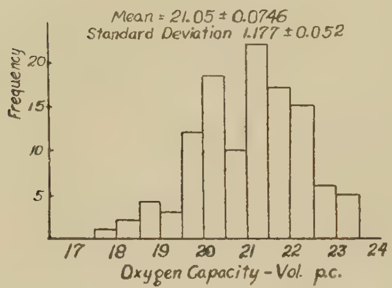

Fig. 1.

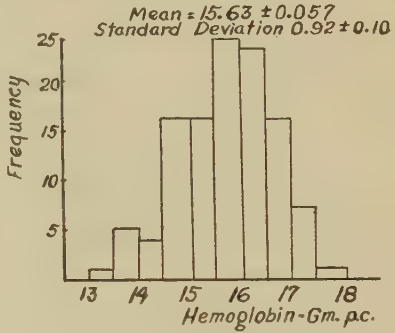

Fig. 2.

The hemoglobin content was calculated from the oxygen capacities and is expressed in Fig. 2. Haldane,[4] accepting the results of Hüfner, regarded 18.5 cc. of oxygen as equivalent to 13.8 gm. of hemoglobin, or 1.34 cc. of oxygen = 1 gm. of hemoglobin. This figure is the one accepted today and is supported by the fact that 1 atom of iron in the hemoglobin molecule unites with 2 atoms of oxygen[6] and that the molecular weight of hemoglobin is a multiple of about 16,750, each unit containing 1 atom of iron.[7]

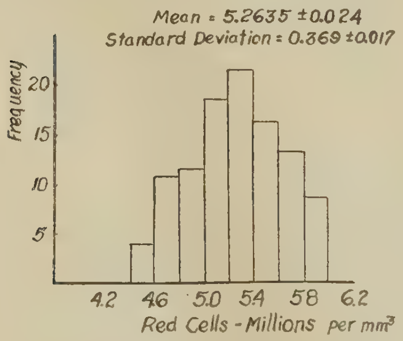

Fig. 3.

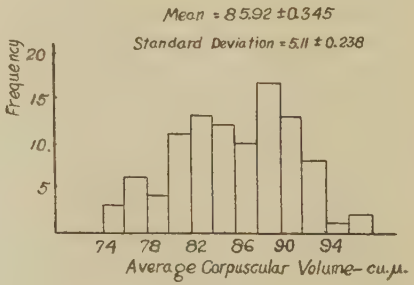

Fig. 4.

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[5] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

[6] Peters, R. A., *J. Physiol.*, 1912, **44**, 131.

[7] Adair, G. S., *Proc. Roy. Soc.*, 1925, 109A, 292.

Red cell counts on 100 of the subjects were made by 2 observers checking within 0.2 million. Oxalated blood was used after first finding that it checked with determinations on fresh blood. See Fig. 3.

Hematocrits were made on the same 100 subjects by the Van Allen[8] method. Ponder[9] has shown that no hematocrit, and particularly one like the Van Allen, where the blood is first diluted with an "isotonic fluid," is above question. We chose the Van Allen method because it is simple, because it gives good checks, and because we found that it can be used with oxalated bloods without the necessity of making corrections. The average on 20 subjects using freshly drawn blood was 44.3 p.c. and on oxalated blood which had stood up to 3 hours was 44.7. Since blood is diluted about 50 times in the Van Allen tubes, the tonicity due to the initial addition of 0.2 p.c. sodium oxalate is negligible. The following table shows that the Van Allen method gives lower results than are obtained on centrifuging undiluted heparinized blood. It will be observed that about 8.5 p.c. of the observed reading must be added

TABLE I.

Conditions		Hematocrit*	Av. Corp. Vol.*
Van Allen method	30 min. at 2200 RPM	44.4	84.4
Heparinized blood 100 × 4 mm. tubes	45 " at 2800 RPM	46.7	88.8
Oxalated blood 100 × 4 mm. tubes	45 " at 2800 RPM	42.9	81.5

*Average of 40 subjects. Red count = 5.26. Hb = 15.74 mg.

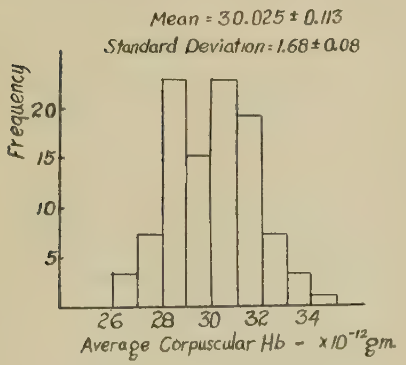

Fig. 5.

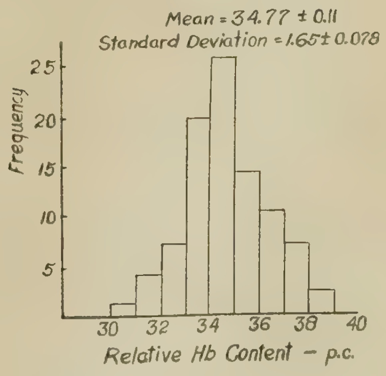

Fig. 6.

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[8] Van Allen, C. M., *J. Lab. Clin. Med.*, 1925, **10**, 1027.

[9] Ponder, E., and Saslow, G., *J. Physiol.*, 1930, **70**, 18.

to the oxalated blood to make it equal the observed reading of the heparinized blood.

The average corpuscular volumes were calculated and the results expressed in Fig. 4. Doubtless the wide variation in our results was in part due to errors in the hematocrit determinations, the true range by our method probably lying between 80 and 92 cu. μ.

The average hemoglobin content of the red cells is shown in Fig. 5. The relative hemoglobin content of the cells is shown in Fig. 6. This is obtained by dividing the hemoglobin content of the cell by the volume of the cell.

5603

X-Ray Studies of Motility of Gastro-Intestinal Tract of Rachitic Rats with Healed Bone Lesions.

LEON J. MENVILLE, J. N. ANÉ AND S. N. BLACKBERG.

From the Department of Medicine, Tulane University, and the Department of Pharmacology, Columbia University.

We previously reported certain observations on the motility of the gastro-intestinal tract of a large number of normal and rachitic rats. It was found that the motility of the gastro-intestinal tract of rachitic rats was markedly altered, and in every case we found a hypomotility.[1]

This study was undertaken to ascertain whether the motility of the gastro-intestinal tract of rachitic rats returned to normal after the bone lesions of the rats were healed.

Three young rats of the same age were fed a rachitic diet for a period of 4 weeks, at the end of which time typical rachitic bone lesions were found in each animal. Gastro-intestinal examinations demonstrated a hypomotility of the gastro-intestinal tract.

TABLE I.

Rats	Stom. Emp. Time	Sm. Int. Emp. Time	Colon Emp. Time
3 rachitic rats	7 hrs. 25 min.	8 hrs. 25 min.	90 hours
The same rachitic rats healed with Viosterol	7 " 15 "	8 " 15 "	88 "
2 normal rats	6 " 18 "	7 " 32 "	65 "

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[1] Menville, L. J., Blackberg, S. N., and Ané, J. N., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 758.

Each of the rats was given 5 drops of Viosterol per day, and the animals were continued on the original rachitic diet. After a period of 10 days roentgen examination showed that the bone lesions of all the rats were healed, but the motility of the gastro-intestinal tract had not been affected. There remained a hypomotility of this tract.

Conclusions. The hypomotility of the gastro-intestinal tract of rachitic rats is evident even after the healing of bone lesions. We appreciate that the number of rats used in our experiment is small, but we have received sufficient encouragement for us to continue on with the work.

Illinois Section.

Billings Hospital, May 26, 1931.

5604

Effect of Hypophysectomy on the Incisor of the Rat.

I. SCHOUR AND H. B. VAN DYKE.

From the Department of Dental Histology, University of Illinois, and the Department of Anatomy and the Pharmacological Laboratory, University of Chicago.

The gross and microscopic anatomy and the rate of eruption of the incisors were studied in 2 groups of rats:

A. Completely hypophysectomized. 16 animals. Age at operation: 34 to 64 days. Age at death: 145 to 459 days. B. Littermate controls. Some were unoperated but most were unsuccessfully subjected to operation. Normal oestrous cycles and normal rate of growth. 9 animals.

Significant alterations were observed only in group A. The incisors of these animals were about two-thirds the size of those of the other groups as judged by planimeter readings of radiographs. In a few of the rats that lived longest after the operation there were seen definite waves on the exposed labial contour of the upper incisors. The rate of eruption was retarded about 7 days after the operation. This retardation was progressive until there was cessation of eruption.

The radiographs of the teeth showed no changes in the size or structure of the molars but indicated very clearly striking disturbances in the form and structure of the rapidly-growing incisors, especially the upper incisors. There were 2 areas to be distinguished in the radiographs and the histologic sections: an anterior zone which comprised about eight or nine tenths of the tooth, and a basal zone which contained the base of the pulp and was the seat of activity of Hertwig's sheath. The tooth substance was practically solid in the anterior zone. The labial contour in the anterior zone was not an arc of a circle but was interrupted by several waves. In the basal zone the labial contour was distorted and presented multiple narrow and deep foldings.

The histologic studies served to confirm and amplify the gross findings. The enamel-epithelium was lacking in the anterior zone and markedly reduced in the basal zone. The enamel was chiefly altered in the basal zone and was often completely absent. The dentin was found almost completely to fill the anterior zone; in the basal zone it showed multiple foldings and was often poorly calcified. The pulp was almost entirely limited to the basal zone and was of reduced vascularity. The cementum was thickened. Regressive changes could also be observed in Hertwig's sheath, the periodontal membrane and the labial alveolar periosteum.

No changes were found in the molars which had usually attained their growth before operation.

Intermediate changes were found in an additional group of 5 rats which were apparently partially hypophysectomized as indicated by retarded growth and infrequent oestrous cycles.

5605

Studies on Experimental Jejunal Ulcer.

HILGER P. JENKINS AND WALTER L. PALMER.

From the Departments of Surgery and Medicine, University of Chicago.

In this paper we are concerned with the experimental production of jejunal ulcers by the "duodenal drainage" method described by Mann. This consists of resection of the pylorus, closure of the upper end of the duodenum, section of the jejunum just beyond the ligament of Treitz, anastomosis of the proximal loop of the jejunum to the ileum, and an end-to-end anastomosis of the distal loop to the end of the stomach. The only striking difference between the method used here and that of Mann was that a side-to-side gastroenterostomy stoma was made instead of the end-to-end anastomosis that he used. The duodenal secretions along with bile and pancreatic juice were drained into the ileum or jejunum at varying levels instead of close to the caecum as he described.

In a series of 12 dogs, 2 died in 13 and 25 days from complications without ulcer formation. One animal is alive one year after the operation apparently in good health. Nine animals died and a definite ulcer was found in the jejunum. Of these 7 died from peritonitis following perforation of the ulcer and 2 from incidental infections. The ulcers were located on the mesenteric side of the jejunum opposite the end of the stoma in 6 animals, while in 2 the ulcer was located on the anti-mesenteric side just beyond the stoma. In one animal there were 2 ulcers found located in the 2 positions described. The size of the ulcers varied from a diameter of .7 cm. to an oval shaped one measuring 2.5 x 3.2 cm. The size of the stoma varied from 4.5 cm. to 12 cm. in circumference. The average circumference was 7.42 cm. The shortest duration of life in which an ulcer was found was 32 days. The longest duration was 228 days. The average length of life was 100 days. The point of drainage varied from the relatively shortest distance from the caecum of 75 cm. to as high as 175 cm. from the caecum. Weight loss was observed in all the animals, which varied from 2.8 kg. to 9.2 kg., or an average of 5.4 kg., except the animal which is still alive and has gained 5 kg. since operation. Gastric analyses made on most of the animals demonstrated an acidity which did not deviate strikingly from the normal.

Conclusions. 1. When the "duodenal drainage" procedure described by Mann was modified by the use of a wide gastroenterostomy stoma instead of the narrow end-to-end anastomosis, jejunal ulcers developed in 90% of the animals which survived the operation one month. The lesions were located opposite the end of the stoma or just beyond it, not at the suture line. It seems to us that the possibility of mechanical injury of the jejunal mucosa due to the ejection of chyme from a narrow stoma is not very great, because these lesions were obtained with a large stoma. This experiment apparently emphasizes the importance of the chemical action of unneutralized gastric juice on the jejunal mucosa. 2. The point of drainage of the duodenal secretions into the small bowel did not appear to markedly alter the formation of ulcers except that the animals which had a relatively high implantation survived longer. The animal with the highest implantation is still alive and in good condition one year after the operation.

5606

Protective and Complement-binding Bodies in the Serum of Human Yellow Fever Convalescents.

N. PAUL HUDSON.

From the International Health Division of The Rockefeller Foundation and the Department of Hygiene and Bacteriology, The University of Chicago.

We have had the opportunity to make a serological study of 5 cases of yellow fever due to laboratory infections. The sera of 4 of these were examined for protective and complement-fixing bodies throughout convalescence so far as was practicable, while the serum of the other case was examined for the first property from the seventh week and for the second property from the eighth month after the onset of illness.

Protective antibodies: The technic of testing for the protective property was that usually employed, namely, the intraperitoneal inoculation of monkeys with serum, followed after 6 hours by the subcutaneous injection of fresh monkey blood containing virus. The table giving the results of these tests indicates that antibodies protecting monkeys against massive doses of virus appeared about the fifth day of illness in 4 cases. In the one instance in which serum was examined for the first time 7 weeks after illness, this protective property was present and repeatedly demonstrated thereafter.

Complement-binding bodies: The technic we employed in the complement-fixation tests was as described in a recent publication,[1] where the literature is briefly reviewed. It consisted essentially in the use as antigen of pooled infectious monkey serum preserved in a dry state, in the employment of a series of antigen dilutions against a constant dilution of serum, and in overnight fixation at 5°C. The usual controls were always included and in the experiments reported here were satisfactory.

A positive reaction appeared in one case in the eighth week after the onset of illness, and in another in the ninth week. The sera of 2 individuals gave positive tests at their first examination, 4 and 8 months after onset, while the reaction with the serum of one person has been consistently negative during the 8 months of observation after the acute attack. The same antigen preparation was used throughout these tests conducted for more than a year.

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[1] Hudson, N. Paul, in press.

TABLE I.
Serological reactions of human yellow fever convalescents.

Protective antibodies

Days after onset of illness			2	3	4	5	6	7	8	Remarks
Case No.		Before illness								
1	(W. A. S.)	not tested	—	—	—	—	—	—	—	Repeatedly positive from 7 weeks after illness.
2	(K. S.)	not tested	—	neg.	neg.	pos.	—	—	pos.	Repeatedly positive later
3	(N. P. H.)	negative	—	neg.	—	—	pos.	—	pos.	
4	(G. C.)	not tested	neg.	—	neg.	—	—	pos.	—	
5	(S. F. K.)	negative	—	neg.	—	pos.	—	pos.	—	

Complement-fixation

Weeks after onset of illness			2	5	8	9	10	12	13	19	
Case No.		Before illness									
1	(W. A. S.)	not tested	—	—	—	—	—	—	—	—	Positive from 8 to 21* mo. after illness.
2	(K. S.)	not tested	neg.	neg.	—	pos.	pos.	—	—	—	Positive for 9* mo. after illness
3	(N. P. H.)	negative	—	—	—	neg.	—	neg.	—	—	Negative for 8* mo. after illness.
4	(G. C.)	negative	neg.	—	pos.	—	—	—	—	—	Repeated positive tests on the one specimen.
5	(S. F. K.)	negative	—	—	—	—	—	—	weak pos.	pos.	The only specimens examined.

* = the time limit of experimentation.

Further laboratory and clinical studies on these cases are to be reported by Berry and Kitchen,[2] and the numbers of the cases given in the accompanying table are made to conform to their case numbers. Our findings in regard to the protective and complement-fixing properties cannot be correlated with the severity of illness of the patients.

The appearance of positive fixation reactions later than protective antibodies is probably due either to the crudeness of the complement-fixation test as conducted, or to the result of persistence of the virus in the host, or to the combination of both factors. When a positive fixation reaction occurred, it has persisted to the time limit of experimentation, in one instance to 21 months after onset of illness.

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[2] Berry, G. P., and Kitchen, S. F., to be published.

The animal test for protective antibodies as a means of diagnosis is expensive and not without danger to the worker. If the complement-fixation reaction is developed so as to give a high proportion of reliable results without false positives, it appears essential to apply it not before several weeks after the suspected illness.

5607

The Cardiac Output in Hyperventilation.*

N. W. ROOME. (Introduced by D. B. Phemister.)

From the Department of Surgery, University of Chicago.

Henderson[1] described the depression of blood pressure in acapnia, advanced the hypothesis of a venopressor mechanism, and still believes that the decrease of the venous return to the right heart is a principal factor in the production of this effect.[2] Dale and Evans[3] showed by cardiometer experiments that the cardiac output is not significantly altered in hyperventilation, and thought the effect due to depression of the vasomotor centres of the bulb and spinal cord. Voluntary over-breathing in man results inconstantly in a reduction of the blood pressure; and McDowell[4] reported that in some dogs anesthetized with chloralose no fall, or even a rise, of blood pressure occurred with acapnia, while in all animals anesthetized with ether a fall occurred. These observations were explained by the hypothesis that acapnia has a dual effect on the circulation, (a) vasodilatation due to a central action, and (b) constriction of smaller and more peripheral vessels due to a local action.

In studying the effects of acapnia, it was desired to measure by the Fick method the cardiac output before and during hyperventilation, and to compare the effect on the output with the effect of partial obstruction of the venous return to the heart.

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* This work was done under a grant from the Douglas Smith Foundation of the University of Chicago.

[1] Henderson, Y., *Proc. Am. Physiol. Soc.*, Dec., 1905.

[2] Henderson, Y., *J. Am. Med. Assn.*, 1930, **95**, 572.

[3] Dale, H. H., and Evans, C. L., *J. Physiol.*, 1922, **56**, 125.

[4] McDowell, R. J. S., *J. Physiol.*, 1930, **70**, 301.

Dogs of 5 to 8 kgm. body weight, anesthetized with sodium-barbital, were used in all experiments. Ventilation of increased rate and volume was obtained by alternate positive and negative air pressure applied to the entire body except the head, in the manner of the Drinker respirator. A cylindrical metal chamber, 35 cm. x 15 cm. in diam., was used; its open end was closed by a thick rubber cuff, sealed about the dog's neck by several layers of adhesive tape. The chamber was connected by a side-arm to 2 Palmer respiration pumps, operated synchronously by an electric motor, at 115 or 160 r.p.m. Oxygen consumption was measured by a Benedict type spirometer. The spirometer could not freely follow rapid respiratory movements; hence a breathing chamber with one wall a rubber diaphragm was devised to take up the oscillatory movement, while the position of the float continued to indicate the oxygen consumption. The chamber used was a metal cylinder, 19 x 22.5 cm. diam., connected directly to the tracheal cannula, and containing soda-lime on its floor and in a gauze bag suspended over the tracheal connection. Air was continuously circulated through the whole apparatus by a motor blower. Samples of the mixed venous blood were obtained from the right heart by means of a metal cannula 20 cm. long, passed down the right jugular vein; samples of arterial blood were collected through a T-cannula in the right carotid artery. The arterial blood pressure was recorded by a manometer connected to a cannula in the left carotid artery. Sufficient heparin (Howell) was given intravenously to render the blood incoagulable. Calculations of the cardiac minute volume were by the Fick formula, the arterio-venous oxygen difference being estimated by means of a Van Slyke manometric blood-gas machine.

It was found that in hypocapnia the blood pressure fell proportionately more than the cardiac output. In 3 dogs, the cardiac minute volume remained at 90 to 110%, while the blood pressure fell to levels 72.5 to 83% of the normal. This contrasts with the effect of obstruction to the venous return by ligation of various large veins, when the output of the heart is diminished proportionately more than the blood pressure; in one dog the cardiac output fell 78.5% while the blood pressure remained at the normal level, and was 41% at a blood pressure level 67% of the normal.

These findings confirm the opinion that the fall in blood pressure in hyperventilation occurs without much depression of the circulation, and is probably due to an arteriomotor relaxation.

5608

Isolation of Histamine from the Pyloric Mucosa.

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From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Although histamine has been isolated from the intestinal mucosa,[1] and from the combined gastric and intestinal mucosa,[2] its isolation from the gastric mucosa alone has not been reported. This report deals with the isolation of the substance in crystalline form, from the pyloric mucosa of the hog, under conditions which preclude the possibility that it is present as a result of putrefaction.

The material was obtained at the slaughter house within 30 minutes after the animal was killed. The mucosa was washed with water and immediately subjected to extraction by 1% sulfuric acid in 80% alcohol. Activated charcoal was added to the extract in sufficient quantity to remove all pigment, the extract filtered from the charcoal, and the histamine adsorbed on Lloyd's Reagent (hydrous aluminum silicate). The Lloyd's Reagent was then extracted with dilute aqueous ammonia, which liberated the histamine. The ammoniacal solution was evaporated to a small volume and mixed with 1½ times its weight of anhydrous sodium carbonate. On standing overnight in a dessicator, this formed a dry cake. This was powdered and extracted with chloroform in a continuous extraction device, until fresh chloroform extracts no longer gave a Pauly reaction. The combined chloroform extracts were filtered from some gummy substance and extracted with a small amount of water. The aqueous extract was boiled to remove chloroform and volatile bases, and then submitted to fractional precipitation with silver nitrate and barium hydroxide, according to the method of Kutscher.[3] The histamine was found in the fraction precipitated by silver in alkaline solution.

The silver precipitate was decomposed with dilute hydrochloric acid and the solution evaporated to a small volume. On standing, a crystalline material separated. This was removed by filtration and the filtrate concentrated to a thin syrup. To this, 20 volumes of alcohol were added, then concentrated sulfuric acid was added, drop by drop, to maximum precipitation. The crystalline precipitate of histamine sulfate was separated by filtration and washed with alcohol and ether.

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[1] Barger and Dale, *J. Physiol.*, 1911, **41**, 499.

[2] Abel and Kuboda, *J. Pharm. Exp. Therap.*, 1919, **13**, 243.

[3] Kutscher, quoted by Barger, "The Simpler Natural Bases," London, 1914, 120.

For identification, the histamine was converted into the dipicrate. The rhombic yellow leaflets obtained melted at 230-232°C. The melting point was unchanged when the material was mixed with dipicrate from known histamine.

5609

Experimental Low Colonic Obstruction.

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From the Departments of Medicine and Physiology and Pharmacology, Northwestern University Medical School.

The evidence has been accumulating that one of the major factors in death from high intestinal obstruction is loss of fluid and chlorides. This does not signify that a toxemia of unknown nature is not important in such cases. For this reason we have studied a series of dogs with low colonic obstruction. Eleven dogs survived the operation and at autopsy showed no cause of death other than simple obstruction; in 5 of these, obstruction was produced by a band of gauze tied tightly about the rectum; in the remaining 6 the colon was severed about 8 inches above the anus and both ends closed with sutures. The average length of life in these 12 animals was 8½ days, maximum 20 and minimum 5 days. Autopsies showed the large bowel much dilated, discolored, and filled with gas and fluid fecal material. The small bowel was also markedly distended for some distance above the ileo-cecal sphincter, and showed hemorrhagic areas in the lower portion. The gall bladder was usually dilated; peritoneal cavity clear and containing little or no free fluid.

Food and fluid were allowed ad lib. Some of the animals ate well up to a few days before death, others refused food from the time of operation until death occurred. The 2 showing longest survival periods (19 and 20 days) ate almost like normal animals. All of the animals vomited slightly, the amount depending upon the intake and increasing in the 48 hours prior to death. In no case was the vomiting abundant, as seen in high obstruction.

Plasma chlorides were followed in all animals. In only 2 was a significant fall observed; these animals lived 5 and 6 days, respectively, and vomited greater quantities than the others of the series. In one the chlorides fell from a control level of 460 to 338, in the other from 552 to 445. In none of the remaining animals did the chlorides fall more than 50 mg./100 and in some cases the level just before death was higher than the control.

Blood urea was studied in 5 of the animals and in all showed a steady rise, in one case reaching 60 mg./100 the day before death. Qualitative analyses for blood indican were also made in 5 animals; the amount of indican increased only slightly above the control level.

In 3 dogs 100 cc. of barium suspension was given as an enema just prior to operation. The colon was occluded with the barium in place, and the position of the barium was ascertained each day by fluoroscopy. In no case was the barium observed to pass above the ileo-cecal valve although in each dog it appeared to mix uniformly with the contents of the colon. This indicates that the fecal matter which these dogs may vomit in small amounts probably is not brought up by reverse peristalsis from the colon.

In these dogs loss of chlorides does not appear to be an important factor, as it is in high obstruction. At the same time, the larger area of absorbing surface presented to the contents of the obstructed bowel may increase the absorption of toxic material. Thus while in high obstruction loss of chloride and fluid may play the predominant role, in low obstruction the part played by chloride loss is overshadowed by some other factor, probably absorption of toxic substances. It has been shown that distension,[1] such as occurs in these animals, may increase such absorption. These results suggest that absorption of toxins cannot be ignored as a cause of death in simple obstruction.

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[1] Dragstedt, C. A., Lang, V. F., and Millet, R. F., *Arch. Surg.*, 1929, **18**, 2257.

5610

The Etiology of Gall Stones. I. Chemical Factors.*

RUDOLF SCHOENHEIMER AND LEO HRDINA.
(Introduced by Edmund Andrews.)
From the Department of Surgery, University of Chicago.

The problem of gall stone formation is the problem of the precipitation of cholesterol out of the bile. An explanation is only possible when we know how the cholesterol is held in solution in the bile. The bile differs from all the other body fluids in that it contains large amounts of bile salts which, for a long time, have been known to have the power to hold many ordinarily insoluble substances in solution. Long ago Wieland and Sorge[1] showed that desoxycholic acid, a split product of the glyco- and taurodesoxycholic acids occurring in bile formed with various substances, insoluble in water, a soluble addition compound. We have investigated the question as to whether one certain bile acid or all bile acids are responsible for the solubility of the cholesterol in the bile.

We have found that when bile acids are precipitated from human gall bladder or fistula bile by $FeCl_3$, lead acetate by acidification, or by any other means there is a simultaneous precipitation of cholesterol. This can easily be proved by dissolving the bile acid fraction in alcohol and adding digitonin, which brings down a precipitate of cholesterol-digitonid. The complex of bile acids and cholesterol is readily soluble in water and may be purified by various methods without lessening the cholesterol content. These substances have about the same cholesterol content, 6-9%, without regard to the form of bile acids from which they are made.

These same products may be prepared from pure bile acids. We have made these complexes from cholic, desoxycholic acid, glycocholic and taurocholic acids. The properties of these substances are very similar. The bond between the cholesterol and the bile acid is a very loose one and even in the preparation there is very often a slight loss of cholesterol. The bond is considerably looser than in the complex between fatty acids and desoxycholic acid described by Wieland and Sorge.[1] Splitting is brought about for example by digitonin and by many organic solvents (e.g. ether, chloro-

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Wieland, H., and Sorge, H., *Z. f. physiol. Chem.*, 1919, **97**, 1976.

form, etc.) Further investigations are necessary to show whether this is a chemical combination or simply a phenomenon of simultaneous solution. Part of the bile acids yield this complex only in salt form; others, the taurocholic acid, as acids as well.

Large amounts of these complexes have been isolated from human bile. It appears that all or nearly all of the cholesterol is bound to the bile acids in this form. On the contrary in dog or ox bile, enormous volumes yielded but small amounts of the complex, most of the bile acids being in a combination with fatty acids.

The importance of this for human gall stone formation is shown by the slight influences which bring about a breaking up of this complex. If the watery solutions of the Na-glycotholic-acid complex are dialysed, the bile salts pass through the membrane and the cholesterol is retained quantitatively. As there is no longer sufficient bile salts within the membrane to hold the cholesterol in solution it is precipitated. We have demonstrated this phenomenon not only in substances prepared from liver and gall bladder bile ,but in many complexes of bile salts and cholesterol synthetically prepared.

All these experiments show that as soon as the bile acids are got out of the solution, the cholesterol must be precipitated. Any time that the organism by any pathological changes, brings about a significant lowering of the bile acids in the gall bladder in relation to the amount of cholesterol in it, cholesterol precipitation is bound to ensue. The fact that dog and ox bile contain very small amounts of this complex offers an explanation of why cholesterol containing gall stones are never found in animals.

5611

The Etiology of Gall Stones. II. Rôle of the Gall Bladder.*

EDMUND ANDREWS, RUDOLF SCHOENHEIMER AND LEO HRDINA.

From the Department of Surgery, University of Chicago.

In the preceding paper it was shown that the greater content of cholesterol in human bile than in that of animals was associated with the formation of a water soluble complex with bile salts. By this means more cholesterin is held in solution. It is obvious therefore that the maintenance of the cholesterol in solution is dependent on the concentration of bile salts in the bile and that if it falls below a certain level the cholesterol will be precipitated. The following studies of the differential absorption rate of bile acids and cholesterol seem to show that the gall bladder under certain conditions may play the major part in gall stone formation.

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

It will be seen that under normal conditions the gall bladder mucosa in its action in concentrating the bile is able to absorb water, of course, rapidly, and the bile salts and cholesterol, if they are absorbed at all, are absorbed at the same rate. In other words the cholesterol content of the bile rises no faster if as fast as the bile salt content, so that there is a wide margin of safety, and no danger that there will not be enough bile salts to hold the cholesterol in solution.

When the gall bladder is diseased a diametrically opposite action takes place. In this series, the cystic duct was ligated which, as we have shown,[1] leads at once to the infection of the gall bladder. Such a gall bladder with the added insult of the insertion of a needle and ligature of the needle hole looks grossly thickened and diseased at the end of 24-48 hours. In some of the experiments it was also traumatized by scraping its mucosa. In this series of experiments an entirely opposite differential absorption took place. In every case samples taken 24 hours later showed an increase in the concentration of cholesterol of 2 to 85%, average +29% and a lessening in the concentration of the bile salts of 3 to 69%, average —28%. The ratio of bile salts over cholesterol was 97 for normals, 59 for the 16 experiments. This is of a high enough degree to cause the precipitation of the cholesterol if continued for any length of time. In the traumatized gall bladders this phenomenon was still more marked.

In view of the previously reported precipitation of cholesterol from bile acid solutions by dialysis, it seems reasonable to assume that the gall bladder mucosa has a great absorptive power for bile salts if diseased ,and that in the latter case the bile acids are absorbed faster as they would be from any semi-permeable membrane on account of their greater solubility.

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[1] Andrews, E., and Hrdina, L., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 116.

5612

Etiology of Gall Stones. III. Bile Salt-Cholesterol Ratio in Human Gall Stone Cases.*

EDMUND ANDREWS, RUDOLF SCHOENHEIMER AND LEO HRDINA.

From the Department of Surgery, University of Chicago.

If, as has been suggested in a previous paper, cholesterol stones are formed in the human gall bladder because there were not enough bile salts present to hold the cholesterol in solution, one would expect to find in human gall bladders with cholesterol stones a lesser quantity of bile salts. The accompanying table amply confirms this expectation and corroborates the findings of Newman.[1]

TABLE I.
Human Gall Bladder Bile in Cholelithiasis.

Reg. No.	Cholesterol	Bile Salts	B.S./Chol. Ratio
34963	69	1130	16.4
32259	78	175	2.2
3971	138	1302	8.8
33293	114*	0	0.
35576	138	272	2.0
34904	188	1310	7.0
35861	96	735	7.6
37847	282*	0	0.
12869	360*	45	0.12
Average	162	556	3.4
Mixed bladder bile from 30 stone cases postmortem	400	247	0.6

Newman's studies were all upon autopsy material, and for that reason might have been questioned. The first series here given are all on fresh surgical specimens and hence not open to the criticism of agonal changes.

As controls in these studies it is of course impossible to obtain bile from normal human gall bladders, and the autopsy findings of Newman must serve. In his series the controls from normal human gall bladders gave a bile acid-cholesterol ratio of from 10 to 24, and his lowest figures of bile acid content of normal gall bladders were nearly double our highest one in a stone containing gall bladder. As a further control the figures cited by Hammarsten on 2 cases of

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Newman, C. E., *Beitrage Z. Path. Anat. u. z. Allgem. Path.*, 1931, **136**, 187.

sudden accidental death are interesting. His analyses showed ratios of 10 and 40 respectively and the total bile acids were 8.2% and 8.7%, approximately 16 times as high as the average of our operative cases.

It is further very interesting to note that in several of our specimens the bile contained a large amount of cholesterol crystals and those cases had little or no bile acid in the bile. This was true not only in 2 cases with little or no pigment in the bile as a result of long cystic duct obstruction, but in another bile which appeared absolutely normal grossly except for the presence of cholesterol crystals and on chemical examination proved to contain but 42 mg.% of bile salts.

These figures are offered as further proof of the above annunciated theory that cholesterol stones are due to a faulty differential absorption of bile acids and cholesterol by the abnormal gall bladder mucosa.

5613

Intestinal Absorption of Viable Yeast.

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The fate of yeast ingested by mouth has been investigated in this laboratory for the past 2 years. The relative destruction within the lumen of the stomach, small intestine and large intestine has been reported (Montgomery, Boor, Bergeim and Arnold).[1] We wish to report upon the passage of yeast through the wall of the intestinal tract into the body. The procedures carried out by Nedzel[2] for absorption of bacteria have been followed in these investigations.

Fasting dogs were given one cake of compressed yeast (Fleischmann) in 100 cc. saline by a stomach tube and animals were killed at periods indicated in Table I. One gram masses of certain organs were removed and cultured in maltose acid broth. Subcultures were made on maltose acid agar after 24 hours and the presence or absence of yeast was determined as indicated in Table I. In 6%

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[1] Montgomery, Boor, Arnold and Bergeim, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 589.

[2] Nedzel and Arnold, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 358, 360, 361, 364, 366.

TABLE I.
Distribution of viable yeast in organs of dogs after oral administration. Positive cultures in percent of animals used are indicated.

Organ	½ hr. (5 dogs)	1 hr. (6 dogs)	2 hrs. (7 dogs)	4 hrs. (4 dogs)
	%	%	%	%
Liver	40	33	29	25
Lymph	20	33	29	25
Lung	—	—	—	—
Spleen	—	—	—	—
Kidney	—	16	29	25

TABLE II.
Viable yeast in portal blood and bile. Dogs fed yeast by mouth.

Time min.	Specimen	Dog No.					
		I	II	III	IV	V	VI
10	Blood	0	0	0	—	—	—
	Bile	0	0	0	—	—	—
20	Blood	0	0	0	+	—	+
	Bile	0	0	0	—	+	+
30	Blood	0	0	0	—	—	+
	Bile	0	0	0	—	—	+
40	Blood	0	0	0	—	—	—
	Bile	0	0	0	—	—	—
50	Blood	0	0	0	—	—	—
	Bile	0	0	0	—	—	—
60	Blood	—	—	—	—	—	—
	Bile	+	—	—	—	—	—

+ indicates presence of yeast. — indicates absence of yeast.
0 indicates no material cultures.

TABLE III.
Viable yeast in portal blood and bile after duodenal injection.

Time min.	Specimen	Dog No.			
		I	II	III	IV
10	Blood	—	—	—	—
	Bile	—	—	—	—
20	Blood	—	—	++	—
	Bile	—	—	—	—
30	Blood	++++	—	—	—
	Bile	+	—	+	—
40	Blood	—	—	—	—
	Bile	++	—	—	—
50	Blood	++	—	—	—
	Bile	++	—	—	—
60	Blood	—	—	—	—
	Bile	++	—	—	—

+ represents 1-25 colonies. ++++ represents 150 colonies or more.
— indicates no yeast growth.

of the cases, 2 cc. specimens of portal blood and bile were taken every 10 minutes for an hour and placed in maltose broth. After 24 hours these were subcultured as above. (Table II).

Fasting dogs were anesthetized, the abdomen opened, and one cake of yeast dissolved in about 25 cc. saline injected into the duodenum. Animals were killed at the time intervals shown in the accompanying graph, and one gram specimens of the organs removed and treated as in Part I. Two cubic centimeter specimens of portal blood and bile were taken on the dogs every 10 minutes for an hour after injection and cultured as in Part I. Results are shown in Table III.

Dogs, fasted for 24 hours, were anesthetized, the abdomens opened, and amounts of yeast in saline suspension, varying from 10 billion to 300 million cells, injected into the portal vein. The dogs were killed at one half and one hour, as indicated in Tables IV and V, and one gram specimens of the organs shown in these tables cultured in maltose acid broth. Subcultures were made after 24 hours, on maltose agar, and the number of viable yeast determined as indicated in Tables IV and V.

TABLE IV.
Distribution of viable yeast in dogs injected into portal vein.
Dogs killed ½ hour after injection.

Dose, cells in millions	Date	Liver	Lymph	Lung	Spleen	Kidney
10,000	11/20	++++	++	+++	+	++
10,000	11/21	+++	++	+	+	++
4,000	11/24	++	—	—	—	—
3,200	11/25	++	—	—	—	—
2,400	12/19	++	—	—	—	—
720	11/28	+	—	—	—	—

+ represents 1-25 colonies. ++++ represents 150 colonies or more.
— indicates no yeast in organ culture.

TABLE V.
Distribution of viable yeast in dogs, injected into portal vein. Dogs killed 1 hour after injection.

Dose, cells in millions	Date	Liver	Lymph	Lung	Spleen	Kidney
4,200	12/8	1800	90	—	—	—
4,800	12/10	3000	60	3000	40	375
4,800	12/11	65	72	21	—	130
400	12/15	69	—	—	—	—
300	12/17	40	—	—	—	—

When a large dose—5 to 10 billion cells—is injected into the portal vein, the yeast cells are showered throughout all the organs,

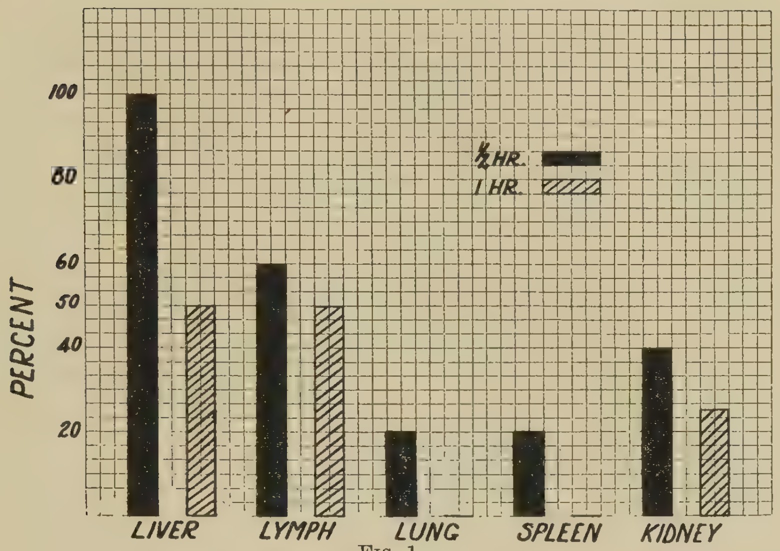

FIG. 1.

Distribution of viable yeast in organs of dogs injected intraduodenally. Positive cultures in percent of animals used are indicated.

but decreasing the dose lowers the incidence of appearance, most of the cells being caught by the liver. Increasing the time interval produces a wider distribution with a smaller dose—1 to 4 billion cells. One accidental finding is of interest; a bitch about 4 weeks pregnant was injected intraportally with 300 million cells and killed within one half hour. Although the dose was much smaller than in previous experiments, yeast was found in appreciable quantities throughout all the organs, indicating that the liver-filtering function was markedly impaired.

Results of human feeding experiments corroborating these results will be published at a later date.

Conclusions. 1. Yeast fed by mouth is absorbed like bacteria into the portal circulation from the lumen of the intestinal tract. 2. Intraduodenal injections did not show increased absorption of yeast over that fed by mouth. 3. Intraportal injections demonstrate that the amount of yeast recovered from the organs, and the relative distribution, varies directly with the amount of yeast injected.

5614

Variations in Self-Disinfecting Power of the Skin During the Menstrual Cycle.

VIRGINIA FISHER. (Introduced by L. Arnold.)

From the Department of Bacteriology, University of Illinois College of Medicine, and Research Laboratories, State Department of Public Health, Chicago.

Several normal young females have been hospitalized and the skin reactions, blood chemistry, and other metabolic studies have been carried out daily for the period of one or more menstrual cycles. We report in this paper the results so far obtained by testing the self-disinfecting power of the skin each day during the complete menstrual cycle.

The technic was the same as that employed by Singer and Arnold[1] and Karns and Arnold.[2] The hands were submerged in suspensions of *B. prodigiosus,* bacteriological specimens were removed from palmar and dorsal surfaces of both hands by means of sterile swabs.[1] Results are an average of the disinfecting power of all 4 surfaces. Patients were tested daily for periods of 28 days.

One studied through 3 menstrual cycles shows a decided inhibition in self-disinfecting power at this time, (A, B and C). The others show daily changes in inhibition, with individual variations as to the time, which may occur during the cycle, (D, E and F). Each individual has a pattern, which is repeated during every cycle; the same general reactions hold for all. Subject Mc—(G) shows a high skin disinfecting power and an interference with menses while upon a ketogenic diet.

The variations in the self-disinfecting power of the skin of the normal female during the menstrual cycle can be definitely correlated with other demonstrable skin changes. Blood chemical and metabolic alterations can also be shown to occur during definite periods of this cycle. The correlation of all of these various tests with self-disinfecting power of the skin will be published later.

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[1] Singer and Arnold, Proc. Soc. Exp. Biol. and Med., 1930, **27**, 364.

[2] Karns and Arnold, Proc. Soc. Exp. Biol. and Med., 1931, **28**, 375.

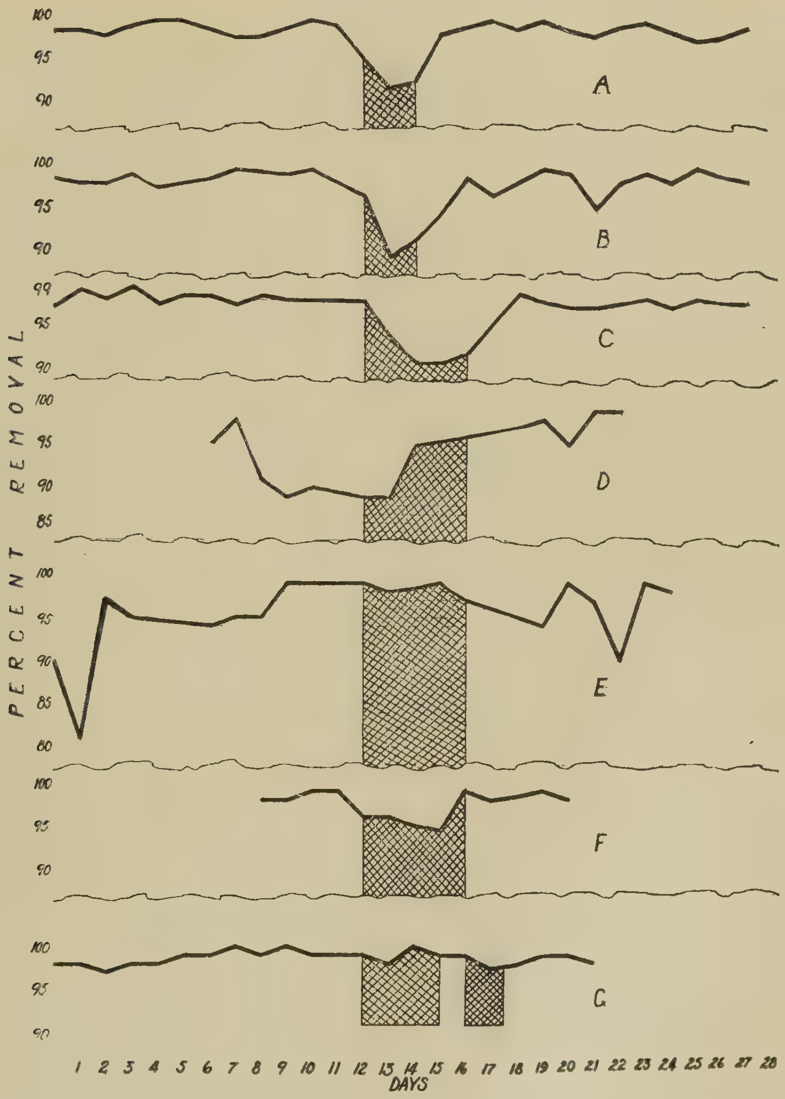

FIG. 1.

Daily determinations of the rate of disappearance of viable *B. prodigiosus* from skin of female subjects during 28 consecutive days. Crossed-in area indicates the menses period.

A, B, C, subject Mc; D, subject D; E, subject L; F, subject F; G, subject Mc, who was on ketogenic diet for two weeks.

5615

Metabolism of Peripheral Leg Nerves and the Ganglionated Nerve Cord of the Lobster.

T. H. CHANG.* (Introduced by R. W. Gerard.)

From the Physiology Course of the Marine Biological Laboratory, Woods Hole.

With the exception of the studies of Meyerhof and Schultz[1] on the oxygen consumption of non-medullated nerve, all recent studies of nerve metabolism have been limited to the usual medullated type. The present experiments, paralleling some of those of Gerard[2] on the frog sciatic, have been carried out on the peripheral leg nerves and the ganglionated nerve cord of the lobster, *Homarus Americanus,* typical unmyelinated nerve of the invertebrate type.

The ventral ganglion chain and the nerves from the large crushing and cutting claws and the small walking legs were carefully dissected out under sea water and kept in this medium throughout the experiments. Oxygen consumption was measured with Warburg manometers, using 3 cc. conical vessels with an inset for alkali and a side bulb for fluid to be poured on the nerve in the course of a run. All experiments were performed at 23.8 to 24.2°C., except those involving the temperature coefficient.

In a number of experiments the dry weight was determined as a fraction of the moist. For the claw nerves it was 11.2% (24 experiments); for the ganglion chain 14.3% (16 experiments). The Qo_2, expressed as cubic millimeters of oxygen per gram moist weight per hour, averaged 81.4 (extremes 48 to 122) for all nerves and 123 (range 107 to 139) for the ganglion chain. The nerves to the cutting or crushing claws each averaged 86; those to the walking legs, 71. No sex difference was observed.

The respiration of the nerves did not remain constant with time, but fell about 10.5% per hour, a little more than this at first, a little less later, during 4 or more hours. The ganglion Qo_2 held surprisingly constant, losing only 1.5% per hour over the same time.

The Q_{10} from 15° to 25° was 1.74 ± 0.1 for the claw nerves (average of 5 experiments).

Addition of methylene blue caused an average increase of 17% in the oxygen consumption of the claw nerves, and 23% for the ganglia. Azure A and B led to about the same rise as the methylene blue. Cresyl blue had a still greater effect, causing an average increase of 60% for the nerves and 29% for the ganglia. The methylene blue and cresyl blue were used in concentrations of about 0.04% and the azure of 0.07%, the dyes being tipped onto the nerves during a run. M/1000 NaCN produced regularly 70% to over 90% inhibition of the oxygen consumption of both tissues, and occasionally inhibition appeared to be complete. This cyanide decrease was largely or completely reversed by the subsequent addition of either cresyl blue or methylene blue. Thus, with cresyl blue, the immediate effect was an increase of oxygen consumption to about 140% of the original level, followed by a fall during 5 hours to a value over 50% of the original. Methylene blue led to a return of oxygen consumption to approximately 50% of the original, both in the ganglion and nerve.

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* The writer takes great pleasure in thanking Prof. R. S. Lillie and Prof. R. W. Gerard for their suggestions and encouragement.

[1] Meyerhof, O., and Schultz, W., *Biochem. Z.*, 1929, **206**, 158.

[2] Gerard, R. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 1052.

Acid production, presumably due to glycolysis, was estimated in terms of the liberation of CO_2 from sea water in a nitrogen filled manometer, and is expressed, similarly to oxygen consumption, as $Q^{N_2}_{CO_2}$. In all cases this value was greatest at the start, fell to 1/2 to 1/5 at the fifth hour, and then apparently began to rise again. The averages for the first eight hours were: leg nerves, 29.7 (7 experiments); claw and leg nerves, 36.7 (6 experiments); ganglion chain, 42.7 (9 experiments). The Q_{CO_2} was increased, in 2 experiments, by the addition of glucose and also by cresyl blue, the nerves responding much more than the ganglia. The oxidation quotients for nerve were 0.42, for ganglia, 0.35; but there is no reason to believe these represent maxima.

Three experiments on *Limulus* claw nerves gave an average Q_{O_2} of 82. The dry weight was 16.5% of the moist.

The actual values of oxygen consumption are somewhat lower than those found by Meyerhof and Schultz for crab nerve at a lower temperature, and it is surprising that the ganglia, including many nerve cells, respire but little more than the nerve itself. The stimulating effect of dyes and their ability to largely offset the inhibition due to cyanide is in harmony with the similar findings of Gerard[2] on the frog sciatic. (Chang and Gerard).[3] Attempts to measure the increased oxygen consumption during stimulation were not entirely satisfactory, and we hope to continue this work later.

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[3] Chang, T. H., and Gerard, R. W., *Am. J. Physiol.*, 1931, **97**, 511.

5616

Difference Between Gonad-Stimulation by Extracts of Pregnancy-Urine and of Pituitary Body.

ZONJA WALLEN-LAWRENCE AND H. B. VAN DYKE.

From the Pharmacological Laboratory, University of Chicago.

The source of the gonad-stimulating substance of pregnancy-urine is still undecided; the weight of evidence, however, indicates that it is extra-hypophyseal in origin. In studies of the comparative effects of extracts of sheep or pork pituitary bodies and of pregnancy-urine or oestrin-free extracts of pregnancy-urine or chorionepithelioma urine, it was found that the minimal effective doses of the urinary preparations were practically the same for both the male and the female. In the cases of crude or refined extracts of pituitary bodies, however, the minimal effective dose for the female was but a fraction (less than 10%) of that required for the male.

Assay was performed on rats exactly 21 days old when the first injection was made; one injection was made daily for 4 days; necropsy was performed at the age of 26 days or 120 hours after the first injection. Assay-results were appraised by the weights of both ovaries or of both seminal vesicles and coagulatory glands. The extracts of sheep and pork pituitary bodies are described elsewhere;[1] urinary preparations were made by repeated alcoholic precipitation or by Dicken's method. Although both the pituitary and the urinary extracts were subjected to various treatments no evidence of the separation of sex-specific gonad-stimulating substances was obtained. Our results indicate either that there are sex-specific gonad-stimulating principles or that the gonad-stimulating principle of the hypophysis is different from that of pregnancy-urine.

The conclusion reached is based upon assays on 352 immature animals (146 females; 206 males).

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[1] Wallen-Lawrence, Z., and van Dyke, H. B., *J. Pharm. Exp. Ther.*, in press.

5617

Subtotal Atelectasis and Pneumectomy in Dogs.

W. E. ADAMS. (Introduced by E. Andrews.)

From the Department of Surgery, University of Chicago.

Permanent closure of the bronchial stump in pneumectomy is essential to a low mortality accompanying that condition. Experimental studies by Bettman[1, 2] and ourselves[3, 4] show healing of the stump to be brought about by peribronchial tissue. Thus, whereas the removal of a lung with a generous stump left for closure is usually attended by success, the reverse is often true when a bare bronchial closure remains.[5]

The instigation of the following experimental work was the finding of a safe and reliable method for closing large bronchi. This consisted of an application of a 35% solution of silver nitrate to the entire circumference of a bronchus. Complete stenosis followed within 2 weeks. With the bronchus stenosed, the lung became atelectatic and its subsequent removal could be safely and easily accomplished.

The following experiments were carried out to determine the maximum amount of pulmonary tissue that could be collapsed or removed in the dog.

In the earlier experiments each bronchus was stenosed individually and only one or 2 lobes were removed at a single operation. Later, however, the right or left primary bronchus was dealt with as a whole and the entire lung removed at one stage. Subsequent stenosis of remaining bronchi was attended with some difficulty, this increasing as more of the pulmonary tissue was collapsed. Once the bronchus was completely stenosed, however, its subsequent removal was always attended by success.

Subtotal Atelectasis and Subtotal Pneumectomy in 6 Dogs. The percentages in Table I represent the value of each lobe in comparison to the total lung volume by weight. Those on the right represent that portion of the total pulmonary tissue functioning at the present time.

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[1] Bettman, R. B., *Arch. Surg.*, 1924, **8**, 418.

[2] Bettman, R. B., *et al.*, *Surg. Gyn. and Obst.*, 1928, **46**, 602.

[3] Adams, W. E., and Livingston, H. M., *Ann. Surg.*, 1930, **91**, 342.

[4] Adams, W. E., and Livingstone, H. M., "A Safe and Sure Method of Closing Large Bronchi." Ready for press.

[5] Robinson, S., and Sauerbruch, F., *Deutsch. Z. f. Chir.*, 1909, **102**, 543.

In 3 dogs, a subtotal atelectasis was produced, while in 3, lobectomy and pneumectomy were performed subsequent to collapse of the pulmonary tissue. No deaths occurred during the experimentation during which time 3 pneumectomies and 2 lobectomies of 2 lobes each were carried out. Following the production of subtotal atelectasis, the dogs became quite dyspneic and somewhat cyanotic, which lasted from 1 to 4 days depending upon the amount of lung tissue collapsed at one time. Subsequent to this acute reaction, they appeared quite normal while at rest but became very dyspneic on slight exertion.

TABLE I. *Pulmonary Lobes.*

Dog No.	15% Rt. Upper	8.7% Rt. Middle	25% Rt. Lower	8.3% Accessory	15% Left Upper	27.9% Left Lower	Lung tissue remaining %
161c	Collapse 6-10-30	Collapse 4-29-30	Collapse 3-4-31	Collapse 6-10-30	Collapse 4-29-31	Normal	27.9
638c	Collapse 5-12-31	Collapse 5-12-31	Collapse 5-12-31	Collapse 6-1-30	Collapse 12-8-30	Normal	27.9
617c	Collapse 10-29-30	Collapse 10-29-30	Collapse 10-29-30	Collapse 10-29-30	Normal	Collapse 5-11-31	15.0
642c	Normal	Collapse 11-26-30 Lobect'y 4-23-31	Normal	Collapse 10-9-30 Lobect'y 4-23-31	Collapse 6-5-30 Pneumectomy 7-1-30	Collapse 6-5-30	40.0
795c	Normal	Normal	Collapse 8-22-30 Lobect'y 8-26-30	Collapse 7-15-30 Lobect'y 8-26-30	Collapse 10-29-30 Pneumectomy 4-23-31	Collapse 10-29-30	23.7
615c	Collapse 10-29-30 Pneumectomy Right 2-19-31	Collapse 10-29-30	Collapse 10-29-30	Collapse 10-29-30	Normal	Normal	42.9

In contrast to this, no dyspnea was noted following lobectomy or pneumectomy. The animals lay quietly in their habitat and refused food for the first 2 or 3 days. Subsequent to this they appeared quite normal with the exception of experiencing a non-productive cough. They, too, exhibited great dyspnea on slight exertion.

5618

Vascular Changes in Chronic Experimental Atelectasis.

W. E. ADAMS. (Introduced by E. Andrews.)

From the Department of Surgery, University of Chicago.

Microscopic pathology of massive atelectasis in man has received little consideration due to its associated low mortality. Reports on this subject have usually been made on autopsies where death was due to some other cause. Bergamini and Shepard[1] reported 2 cases of bilateral massive atelectasis causing sudden death. Microscopic sections of the collapsed lungs revealed cloudy swelling and indistinct outline of the cells; with a dilatation of the capillaries, arterioles and venules which were filled with blood, presenting in parts an angiomatous appearance. From experimental studies it has generally been contended that there appears to be an increased vascularity of the atelectatic tissue; that the capillaries become dilated and engorged with blood simulating a passive congestion of the lung lobe.

Recently we have studied microscopic sections of experimental atelectasis at various stages of duration. Massive atelectasis was produced in dogs by stenosis of the bronchi with a solution of silver nitrate.[2] The dogs were sacrificed at irregular intervals ranging from a few days to several months.

Atelectasis of only a few days' duration revealed complete collapse of the alveolar spaces and smaller bronchioles with some swelling of the septal cells whose outlines were indistinct. There was an apparent increase in the vascularity of the tissue due to the capillary spaces being brought much closer together. These vascular channels appeared to be engorged with blood, giving it the appearance of a passive congestion. Anthracosis was present, scattered pigment appearing throughout the tissue.

By the end of 4 months the alveolar walls were much less closely packed together and the intervening greatly dilated blood spaces more clearly outlined at this time. The blood spaces gradually become larger so that at the end of 9 and 11 months, they were found enormously dilated; some being 2 or 3 times as large as an ordinary alveolus. Accompanying this, the alveolar septae were found fewer in number and widely separated, in contrast to the closely packed alveoli of earlier stages. The tissue in some parts took on the appearance of a cavernous haemangioma. Red blood corpuscles found in these spaces appeared to be in good condition with blood pigment nowhere to be seen.

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[1] Bergamini, H., and Shepard, L. A., *Ann. Surg.*, 1927, **86**, 35.

[2] Adams, W. E., and Livingstone, H. M., *Ann. Surg.*, in press.

Conclusions. In the earlier stages of atelectasis the apparent increased vascularity was partially due to collapsing of the air spaces thus bringing the capillary sinuses in closer proximity. The changes exhibited in the more chronic stages were less easily explained. The process was a gradual one, marked changes being present as early as 4 months which were found to increase with the duration of the disease. That the blood in the dilated spaces was in an active state was strongly suggested by the normality of the red blood cells and the lack of blood pigment. The gradual increase in the size of the blood sinuses was in favor of their being dilated capillary spaces rather than alveoli through which blood was flowing. This last factor is under investigation.

5619

Production of Intestinal Ulcers by Active Gastric Juice.*

WARREN B. MATTHEWS. (Introduced by L. R. Dragstedt.)

From the Department of Surgery, University of Chicago.

Gastric mucosa has[1] a strong natural immunity against corrosion by gastric juice, and wounds made artificially in it tend to heal readily. Mann,[2, 5] Dragstedt and Vaughan,[3] Morton,[4] and others have shown that when other tissues such as kidney, spleen, mucosas of various portions of the intestinal tract with an intact blood supply are implanted into defects made in the stomach wall, they tend to remain sound and healthy without erosion or ulceration, even though exposed to the normal stomach contents. Mann and Williamson,[6, 7] after draining the duodenal juices into the lower ileum, anastomosed the upper jejunum to the stomach, ulcers formed in the jejunum. These ulcers were interpreted as due to the corrosive action of acid gastric contents on a sensitive intestinal mucosa now unprotected by the alkaline duodenal juices, plus the "motor drive" mechanical effect of the stomach contents being ejected forcibly against the spot on the intestinal wall where the ulcer was supposed to form. Ivy and Fauley[8] point out, the emaciation which most of these animals undergo must not be overlooked. Aschner and Karelitz[9] have summarized some 33 cases of ulcer of the ileum associated with Meckel's diverticulum containing heterotopic mucosa of gastric type. These ulcers were interpreted as due to gastric juice secreted by this heterotopic mucosa.

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Ivy, *Arch. Int. Med.*, 1920, **25**, 6.

[2] Mann, *J. Med. Res.*, 1917, **35**, 289.

[3] Dragstedt and Vaughan, *Arch. Surg.*, 1924, **8**, 791.

[4] Morton, C. B., 1927, **85**, 207.

[5] de Takats and Mann, *Ann. Surg.*, 1927, **85**, 698.

[6] Mann and Williamson, *Ann. Surg.*, 1923, **77**, 409.

[7] Mann, *S. Clin. N. Amer.*, 1925, **5**, 753.

We attempted to duplicate this situation experimentally by making a small pawlow pouch, and causing it to empty into an isolated loop of lower ileum, whose other end communicated with the lower ileum just above the ileocaecal valve. In this way the possibility of mechanical action in possible ulcer formation was avoided. The union of pouch with intestinal loop was made by a side-to-side anastomosis, using only 00 chromic catgut for suturing. Clamps were not used on the intestine.

Results. 1. Except for one animal which died at 60 days of distemper, all the animals remained in apparent good health and nutrition until 2 died suddenly of peritonitis from perforation of ulcers, and the other 3 were sacrificed 80 days after the operation. 2. Ulcers formed in the ileal mucosa near or adjacent its union with the gastric mucosa of the pouch in all 6 dogs. The gastric mucosa was never involved. The ulcers were found between 30 and 80 days. All were chronic in type, large (from 1 to 5 cm. across), indurated, with clean, granulating bases usually formed of connective tissue. Grossly and microscopically they appeared similar to so-called "peptic ulcers" in the human intestine.

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[8] Ivy and Fauley, *Am. J. Surg.*, n. s., 1931, **11**, 531.

[9] Aschner and Karelitz, *Ann. Surg.*, 1930, **91**, 573.

Western New York Section.

Clifton Springs Sanitarium, May 23, 1931.

5620

Certain Factors in the Preparation of Cortin.*

F. A. HARTMAN, K. A. BROWNELL AND A. A. CROSBY.

From the Department of Physiology, University of Buffalo.

The potency of the extracts used in this study has been tested by the effect on the growth of young adrenalectomized white rats.[1] This test has been carried out immediately after adrenalectomy as that is the most crucial period of adrenal insufficiency. Sometimes when the animals have failed to grow they have been further tested by their resistance to cold,[2] comparison being made with adrenalectomized rats treated with adequate cortin.

In an earlier work on the hormone of the adrenal cortex in which it was salted out with the globulins[3] we found that exposure of the precipitate to air for a few hours reduced the potency. Moreover heating the extract of the sodium chloride precipitate to 80°C. for 5 min. with stirring, to cause admixture with air, destroyed the hormone.[4] From this evidence we concluded that it was necessary to operate in the absence of O_2. Distillation of the ether at atmospheric pressure in the preparation by the ether-alcohol method[4] produces a less potent extract than when the distillation is *in vacuo.* Heating the concentrated extract in an open beaker for one hour at 80°C. without stirring does not appreciably destroy the hormone. The extract can be boiled for a short period (5 minutes) in the absence of oxygen without great loss of potency.

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* Aided by a grant from the Ella Sachs Plotz Foundation.

[1] Hartman and Thorn, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 94.

[2] Hartman, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 702.

[3] Hartman, Brownell, Hartman, Dean and McArthur, *Am. J. Physiol.*, 1928, **86**, 353.

[4] Hartman, Brownell and Hartman, *Am. J. Physiol.*, 1930, **95**, 670.

Small amounts of antioxidants, such as hydroquinone, resorcinol or levulose retard the loss of potency in aqueous solution. The hormone can be preserved in 80-95% alcohol for weeks. Cortin is very unstable in the presence of the fixed alkalies. It seems to be completely destroyed by tenth normal NaOH at 20°C. in less than one hour. On the other hand tenth normal NH_4OH under the same conditions has little or no effect. Tenth normal HCl at 20°C. for one hour is without effect. Extraction of the dry residue with water for the final product must not be aided by heat nor must it proceed for a long period because toxic substances are absorbed. An extract made by allowing water to stand on the residue for one hour at 45°C. when injected into adrenalectomized animals caused inflammation and loss of weight.

We have made extracts of whole adrenals by many different methods in an attempt to separate cortin from the toxic substances which develop in the medulla.[5] No method has been found which yields as potent an extract per unit of cortex as does the simple method described. Active charcoal seems to remove both cortin and toxic substances from an aqueous solution (pH 8-6.5). Attempts to elute the hormone have been partially successful. Permutit removes toxic substances and some cortin from an alcoholic solution. Lloyd's reagent removes toxic substances together with some cortin from an alcoholic solution. $Al(OH_3)$ acts in a similar fashion.

5621

How Intravenous Infusions Modify the Water Contents of Tissues.

E. F. ADOLPH AND M. J. LEPORE.

From the Physiological Laboratory, University of Rochester School of Medicine and Dentistry.

To find what factors influence the exchanges of fluid between blood and tissues, muscles and arterial blood were sampled at frequent intervals before, during, and after various acute procedures upon anesthetized cats and dogs. The percentage water contents of the muscles, and occasionally of other tissues, were determined, and blood concentration was measured in parallel by several methods.

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[5] McKinley and Fisher, *Am. J. Physiol.*, 1926, **76**, 268.

When 50 cc. of Locke solution per kilo were infused, the muscle water content remained constant for a few minutes, then suddenly increased by 1 to 8% and promptly returned almost to normal. The dilution of the blood was maintained for a much longer period of time. The only factor measured which varied in parallel with muscle hydration was the mean arterial blood pressure. That blood pressure was more important than blood dilution in regulating the water content of tissues was shown by infusions of gelatin-Locke and of acacia-Locke solutions. In these experiments the blood dilution persisted for at least 3 hours, while the muscle water content returned to normal within one hour.

A remarkable lag occurred in the onset of tissue hydration in all infusion experiments. The water content of muscle began to increase only at 8 to 20 minutes after the blood dilution and the rise of blood pressure had been produced. The hydration reached a sharp peak and fell with a lag of about 10 minutes after the fall of blood pressure.

After hemorrhage the muscle became dehydrated, following the blood pressure with the usual lag. The blood, both as a whole and with respect to plasma proteins, became diluted without any lag. In hemorrhage the composition of the blood was being varied only through exchanges with the tissues, and pressure changes evidently initiated these.

The importance of blood pressure alone in governing the water content of tissues was well illustrated by experiments in which the dorsal aorta was partially clamped, producing low pressures in the hindlegs and high pressures in the viscera and foreparts. In each part the hydration of muscle followed closely the change of mean arterial pressure and returned to normal after the clamp was removed.

The blood cannot be said to possess an excess of effective osmotic pressure, but all forces are in dynamic equilibrium during the maintenance of normal water distribution. When the mean arterial pressure increases 20%, muscles gain between 1 and 2% in water content.

It is concluded that the changes of capillary blood pressure which accompany sudden changes of blood volume may be more important than the changes of blood concentration or of colloidal osmotic pressure in controlling the initial exchanges of fluid between plasma and tissues. The time factor in these exchanges indicates that shifts occur in circulatory or other conditions such that water is successively translocated among various tissues. Undoubtedly changes of blood pressure are accompanied by modifications of blood flow which may induce local differences of oxygen tension and consequent changes of permeability or of hydrostatic pressures.

New York Section.

5622

The Nature of the Respiratory Supplement.

K. SALOMON AND L. MICHAELIS.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

When the oxygen consumption of rabbit erythrocytes and that of saline extracts of rat livers (or various other organs), are measured separately, and compared with what happens in a mixture of both, the oxygen consumption is much higher in the mixture than the sum of the oxygen consumptions in the 2 separate systems. This has been shown by the authors previously[1]; the effect of the liver extract has been confirmed by Zeile and Euler.[2]

This paper presents the attempts to elucidate the nature of this reaction. For brevity's sake the active principle of the liver extract will be designated as the respiratory supplement.

1. The supplement deteriorates and may even be destroyed by various mechanical treatments of the liver extract. Extended shaking, in presence or in absence of oxygen damages the supplement. When the extract is centrifuged the supplement activity both of the sediment and of the supernatant fluid is diminished and by mixing the 2 fractions is not restored to its original strength.

2. Several attempts were made to isolate the supplement by adsorption. Sand, Kaolin, Kieselgur adsorb the supplement to only a small extent, according to the amount applied, but never completely. Colloidal iron hydroxyd adsorbed the supplement completely in several experiments, in which the adsorbent was used in an amount sufficient to remove the proteins. Colloidal aluminum hydroxyd removed it completely when applied in so small an amount as to remove only a small part of the proteins. No attempt to re-

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[1] Michaelis, L., and Salomon, K., *J. Gen. Physiol.*, 1930, **13**, 683.

[2] Zeile, K., and v. Euler, H., *Hoppe-Seyler's Z. f. physiol. Chem.*, 1931, **195**, 35.

cover the supplement by elution with N/20 ammonia was successful.

3. Heating to 56° diminishes the supplement considerably though there is no critical temperature for this effect. The inactivation by heat seems to run parallel to the formation of a coarse turbidity or flocculation in the previously slight and homogeneous extract. Freezing did not impair the effect of the supplement at all.

4. The liver extract does not seem to be able to convert hemoglobin to methemoglobin, at least not to a degree detectable with certainty.

5. The supplementary effect of methylene blue (instead of liver extract) first observed by Harrop[3] and Barron[4] and investigated subsequently by Barron,[5] O. Warburg[6] and Wendel[7] is manifested only when the erythrocytes are intact and vanishes in experiments with hemolyzed erythrocytes. This is sometimes the case with liver supplement also as described in our previous paper. However, this phenomenon is not as regular with the liver supplement as with methylene blue. Sometimes the previous laking of the erythrocytes by repeated freezing and thawing, which destroys the effect of methylene blue, has only a slight damaging effect upon the action of the liver supplement.

It is, therefore, evident that the supplement is not simply comparable in nature and action with methylene blue. It has some intrinsic connection with what we may call the colloidal state, or a remainder of some kind of "structure", in the liver extract.

Press juice of liver obtained with Kleinmann's hydraulic micropress and diluted to a volume comparable to that of the usual extract, acts like the extract.

It is noteworthy, furthermore, that the sum of the oxygen consumption of the separated systems (the liver extract and the blood corpuscles) has no noticeable correlation with the oxygen consumption of the mixture. It may happen that the oxygen consumption of each of the components is negligibly small and the oxygen consumption in the mixture is very large.

The reproducibility of the original experiment with liver extract and intact erythrocytes, such as described in the previous paper turned out to be very good. Out of about 75 independent experiments there were only 3 in which the oxygen consumption of the mixture was not at least 3 times that of the isolated components; often it was 10 times that.

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[3] Harrop, G. A., and Barron, E. S. G., *J. Exp. Med.*, 1928, **48**, 207.

[4] Barron, E. S. G., and Harrop, G. A., *J. Biol. Chem.*, 1928, **79**, 65.

[5] Barron, E. S., *J. Biol. Chem.*, 1929, **81**, 445.

[6] Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1930, **227**, 245.

[7] Wendel, W. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 401.

5623

The Enterogastric Reflex.

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Numerous investigators have reported a delay in gastric evacuation as a result of placing acid, fat, gastric juice, etc., in the duodenum or other parts of the small intestine. The effect has generally been attributed to a reflex (the "Hirsch v. Mering reflex") causing tonic contraction of the pyloric sphincter. However, two studies record inhibition of contractions of the *pars pylorica* following the introduction of acid[1] or fat[2] into the duodenum. Apparently both investigations were made upon the empty stomach, though Edelmann[2] observed inhibition of gastric contractions during secretion of gastric juice. There are no data in either report to prove that the phenomenon occurs in the full stomach or has any bearing on the mechanism of gastric evacuation.

We studied the effect of placing various substances in the duodenum while recording graphically the tone changes in the pyloric sphincter by means of the pressure tonometer,[3] and the contractions of the *pars pylorica* by means of a balloon placed in the pyloric canal. Observations were made before, during and after feeding. A balloon in the pyloric canal of the empty stomach registers contractions which are indistinguishable graphically from digestive peristalsis and are probably aroused by the presence of the balloon. We do not consider them hunger contractions. Dogs were used, some narcotized, others without anesthesia or narcosis. The latter were provided with gastric and duodenal fistulas fitted with cannulas.

HCl N/10 in the duodenum, inhibits the contractions of the *pars pylorica* in both the full and empty stomach. In the former the typical effect is decrease in force rather than cessation of contractions. The pyloric sphincter, after a momentary increase in tonus, is likewise relaxed and its rhythmic contractions are inhibited, roughly in proportion to the similar effect on the *pars pylorica.* The effect on both the sphincter and *pars pylorica* is more pronounced when the stomach is empty and is obtained with smaller amounts of acid. In conscious animals with a full stomach the threshold for the inhibitory effect on the stomach is lower than for the primary contraction of the sphincter. In such cases the primary contraction of the sphincter is frequently not observed. With minimal stimuli such as might be expected to occur normally, probably only the gastric contractions are affected. Similar results were obtained using as stimuli gastric contents following a meal of bread with milk or water, hypertonic salt solution, acid sodium phosphate solution, and olive oil. Considerable distention of the duodenum with tap water or isotonic salt solution has a similar, though weaker effect.

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[1] Kirschner, M., and Mangold, E., *Mitteil. a. d. Grenzgeb. d. Med. u. Chirur.*, 1911, **23**, 446.

[2] Edelmann, J., *Jahresbericht f. Tier-Chemie (Malys Jahresb.)*, 1906, **36**, 414.

[3] Thomas, J. Earl, *Am. J. Physiol.*, 1929, **88**, 498.

The relaxation of the sphincter is slight and probably of little consequence, nevertheless it is interesting in view of the fact that contraction has usually been assumed to result from such stimuli as we used. It would appear that the physiologically significant effects of such stimuli are on the force of the gastric peristaltic contractions.

The nervous mechanism of the reflex was studied in dogs narcotized with morphin following ether anesthesia. HCl N/5 was used as a stimulus. The reflex is readily obtained after section of the splanchnics and after tightly ligating the pylorus over a metal plug in the lumen. It was not observed after section of the vagi in the neck. Evidently the inhibitory reflex is mediated by the vagi. We propose to call this the enterogastric reflex. There appears to us to be a rather close analogy between this reflex and the depressor reflex of the heart.

Apparently the same mechanism is involved in the inhibition of hunger contractions by stimuli applied to the intestinal mucosa.[4, 5]

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[4] Boldireff, W. N., *Zentralbl. f. Physiol.*, 1904, **18**, 489.

[5] Brunheimer, E. H., and Carlson, A. J., *Am. J. Physiol.*, 1914, **36**, 191.

5624

Effect of Heat on Mold Spores.

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Because of their wide distribution and ease of dissemination, molds always present a problem in the preparation of foodstuffs that are not to be consumed immediately. The changes produced in foods by molds are in general very undesirable. As heat is the most common way of preserving foods, it was thought desirable to know something about the thermal death times of molds. Thom and Avery[1] found that *Rhizopus nigricans* and *Oidium lactis* were killed in pasteurized milk at 54.5°C. (130.1°F.) in 30 minutes, but that most strains of *Aspergillus* and *Penicillium* resisted this temperature and were killed at 60°C. (140°F.) for 30 minutes.

Since 30 minutes is the customary pasteurization time, and since this experiment was carried out mainly to study the effect of pasteurization, no tests were made at intervals more frequent than 30 minutes. Streider and McClellan[2] studied the thermal relations of molds in bread. They submerged flasks containing mold suspensions in a water bath at 20°C. (68°F.). The temperature was slowly raised to 100°C. (212°F.) so that the elapsed time was 35 minutes. This was done to simulate the temperature obtained when baking bread in the ovens. No molds survived. Loaves of bread that had been exposed to molds were heated to 110°C. (230°F.) at 15 pounds pressure, for 30 seconds, and exposed to live steam for 4 minutes. In the first case, *Rhizopus* and *Aspergillus* species survived, while in the latter, *Aspergillus* species only survived. Complete destruction of the mold was almost attained, as there were very few mold colonies on the plates made from this last experiment. Using the same procedure, loaves were then exposed to dry heat at 120°C. (248°F.) for 10 minutes. Both *Aspergillus* and *Penicillium* species withstood this exposure, although most of the cells were destroyed.

Molds in meat have also been studied. Roderick[3] found that all molds were killed in frankfurters when they were heated in water at 60°C. (140°F.) for 10 minutes. Lewis and Yesair,[4] using temperatures of 50°C. (122°F.), 55°C. (131°F.) and 60°C. (140°F.), exposed pure cultures of molds for periods of time ranging from 5 minutes to one hour. At 50°C. (122°F.) all molds studied except 2 *Aspergillus* species, *Rhizopus nigricans* and *Fusarium,* survived the one hour exposure. Of the latter group they were destroyed in 30, 25, 15 and 5 minutes respectively. When the temperature was raised to 55°C. (131°F.), only *Monilia sitophila* survived the hour exposure. *Mucor racemosus* was destroyed in 25 minutes, *Aspergillus niger* in 20 minutes, *Penicillium expansum* in 15 minutes and *Alternaria tenius* and 2 other *Aspergillus* species in 5 minutes. None of the organisms survived 60°C. (140°F.) for 5 minutes.

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[1] Thom, C., and Ayers, S. H., *J. Ag. Res.*, 1916, **6**, 153.

[2] Streider, J. W., and McClellan, R. N., *Baking Tech.*, 1922, **1**, 230, 282.

[3] Roderick, L. M., 1926. Quoted from Lewis and Yesair.

[4] Lewis, W. L., and Yesair, J., Bull. published by Institute of American Meat Packers, 1928.

In the following study, mold spores from pure cultures were suspended in 1, 3 and 6% salt solutions; 10, 25 and 50% sugar solutions; juice from pitted red cherries in syrup, and distilled water. *Rhizopus nigricans, Mucor mirus, Aspergillus niger, Oidium lactis* and *Alternari solani* were the molds studied. DeKotinsky tubes were filled and sealed in the flame, containing 2 cc. of the mold suspensions. These sealed tubes were submerged in water baths at temperatures of 50°C. (122°F.), 55°C. (131°F.) and 60°C. (140°F.). At 5 minute intervals, a tube of each mold was taken out and submerged in cold water. As soon as cooling was assured, the tubes were wiped with disinfectant, broken and 1 cc. of the contents added to a sterile petri dish for culture.

With *Rhizopus nigricans* the salt solution, cherry juice and distilled water suspensions all acted in a similar way. At 50°C. the organisms were completely destroyed within 20 minutes, and at 55°C. within 10 minutes. In the sugar solution suspensions it took 30 minutes at both 50°C. and 55°C. to completely destroy the cells. The percentage of sugar apparently did not influence the death rate, as the action in all 3 solutions was practically the same. Results of the study of *Aspergillus niger* were very much like those obtained from *Rhizopus nigricans,* except that a slightly higher temperature was necessary. In the salt solution suspensions it took 30 minutes at 55°C. (131°F.). With *Alternaria solani* and *Mucor mirus* the results showed about the same thermal death times as those obtained with *Rhizopus nigricans* and *Aspergillus niger,* except that the salt solution suspensions were the hardest to destroy. Here the salt solution suspensions resisted 50°C. for 30 minutes, but were killed within 10 minutes at 55°C. and the sugar solution suspensions were destroyed within 25 minutes at 50°C. and 5 and 10 minutes at 55°C. In the *Trichothecium* species there was no practical difference between the salt and sugar solution suspensions. It was destroyed within 30 minutes at 50°C. and 5 minutes at 55°C. *Oidium lactis* resisted 60°C. for 30 minutes in all suspensions.

In general, these results agree with those of previous investigators, that, although species of molds vary slightly in their thermal death times, a temperature of 60°C. for 5 minutes is usually sufficient to assure complete destruction. Exceptions were observed in *Aspergillus niger* and *Oidium lactis*. *Aspergillus niger* resisted 60°C. (140°F.) for 10 minutes in some cases, and *Oidium lactis* survived after 30 minutes exposure. From the data it seems that the molds studied react differently towards sugar and salt. In some cases, the sugar protected the organisms more than the salt did, *e. g., Aspergillus niger* and *Rhizopus nigricans;* and in the others, the salt protected more than the sugar did, *e. g., Mucor mirus* and *Alternaria solani*. *Trichothecium* species acted practically the same way in both salt and sugar solution suspensions. As a general rule, the suspensions in distilled water and cherry juice acted very much like those in salt solution. From the former observation, one might conclude that the salt itself was neither harmful nor beneficial to the molds, and consequently, that the sugar exerted a protective action in some cases or a destructive one in others, depending on the nature of the mold. According to Rahn[5] the effect of sugar is protective, the sugar causes a dehydration of the organism, making it more resistant. However, the heat penetration studies of Bigelow[6] lead one to believe that heat penetration might play an important part in this protective action of sugars. The fact that there was no difference between the different sugar solutions, however, presents an argument against this explanation.

5625

The Action of Carbon Filters.

S. P. KRAMER. (Introduced by J. H. Northrop.)

If one filters 100 cc. of a 1% solution of bichloride of mercury through a filter made of 5 gm. of finely divided activated carbon there is no mercury in the filtrate. If one filters through such a filter

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[5] Rahn, O., *Canning Age,* August, 1928, 705.

[6] Bigelow, W. D., *et al.,* Bull. 16 L, Research Lab. Nat. Canners Assn., 1920.

100 cc. of a 1% solution of ferric chloride there is no ferric chloride in the filtrate but 92.5% of the iron is in the filtrate in the form of ferrous chloride. The carbon in both cases has reduced the perchlorides; in the case of mercury the mercurous chloride is insoluble and remains in the filter carbon; in the case of iron the ferrous chloride is soluble and passes through the filter.

Solutions of the toxins of the tetanus, diphtheria and *Botulinus bacilli* respectively were filtered through carbon and in each case the filtrate, when injected into guinea pigs in doses corresponding to 5000 M.L.D. were found to be innocuous. In each case some of the carbon through which the toxins had been passed was injected into guinea pigs.

In the case of the diphtheria toxin-carbon the animal showed no symptoms. In the case of the tetanus and botulinus toxin the animals died with the lesions and symptoms characteristic of the respective toxin.

The tetanus and botulinus toxins are formed under anaerobic conditions and are "immune" to reduction; they are adsorbed by the carbon but not destroyed by its reducing action.

The diphtheria toxin is formed under aerobic conditions and is both adsorbed and rendered innocuous or destroyed by the reducing action of the carbon.

If 50 cc. of a ½% solution of strychnine sulphate is filtered through such a carbon filter the filtrate is free of strychnine to the taste and gives no test for alkaloid, nor does it affect the frog.

The same thing is true of the carbon through which it has passed. One can get no taste, and when injected into the frog in amounts corresponding to a hundred fatal doses, no reaction takes place. Strychnine cannot be recovered from the carbon by acids, alcohol, nor any other solvent.

These filters are made by placing 5 gm. of carbo-raffin, dry, in a glass tube ¾ of an inch in diameter; one end of the tube contains a perforated stopper carrying a glass tube and on the orifice of the tube is a layer of cotton to retain the carbon. The carbon is compressed while still dry by means of the vacuum pump. Filtration is carried on under 750 mm. pressure.

Since this work has been done I have seen the note by Saunders, Schochet and Lackner[1] that strychnine, brucine, adrenalin, tyramine, and histamine were inactivated by carbo-raffin.

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[1] Saunders, Schochet and Lackner, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 564.

5626

Development of the Meninges in the Chick.

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Harvey and Burr[1, 2] have presented evidence that in the salamander, the neural crest was a necessary requisite for the formation of the leptomeninx. We have continued the study by means of chorio-allantoic grafts in the chick. Two series of operations were performed by Dr. Van Campenhout. In the first, the prosencephalon of 50-hour chicks was transplanted adjacent to an allantoic vessel of a 9-day host and allowed to develop from 7 to 9 days. In the second, an entire segment of the thorax or abdomen from a 50- to 98-hour chick was transplanted on the chorio-allantoic membrane of 9-day hosts. These also were permitted to develop for a period of approximately 9 days. Our knowledge of the distribution of the neural crest cells indicates that at the time of operation no such cells have as yet reached the region of the forebrain. It could then be transplanted devoid of neural crest investiture. On the other hand, during the period immediately following the 50-hour incubation period, the neural crest has begun its migration about the spinal cord in the thoracic and abdominal segments. Thus we have the nervous system transplanted without neural crest in the first series and with neural crest in the second. Since all the other conditions were as nearly constant as possible any difference in the structure of the meninges could be attributed to the presence or absence of the neural crest.

The material from the above experimental procedure was prepared for microscopic study using chiefly the Masson stain. A critical examination shows that there is a marked difference in the membranous investment of the nervous system in the 2 series. In series one, without neural crest, the nervous system is surrounded by a series of fibrous laminae of obvious mesenchymal origin. These laminae are in direct contact with the external limiting membrane of the forebrain. All the cellular elements lie outside the connective tissue fibrils. The blood vessels lying adjacent to the nervous system also lie outside this primitive membrane. When blood vessels penetrate the neural wall the connective tissue fibrils tend to pene-

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[1] Harvey, S. C., and Burr, H. S., PROC. SOC. EXP. BIOL. AND MED., 1924, **22**, 52.

[2] Harvey, S. C., and Burr, H. S., *Arch. Neur. and Psych.*, 1926, **15**, 545.

trate beneath the limiting membrane for a short distance. No evidences of fluid spaces could be found about the nervous system. The ventricles were closed systems filled with blood cells and detritus. As a result of this series of transplantations a portion of the nervous system is more or less completely surrounded by a condensation of mesenchyme which has many of the characteristics of a primitive dura.

When, on the other hand, a portion of the nervous system was transplanted together with the neural crest, the relations are quite different. The same condensation of mesenchyme and the beginning of a fibrous sheet occurs as in the first series of transplanted material. But, between the mesodermal sheet and the nervous system, there can now be seen a single or double layer of cells with oval nuclei and scanty cytoplasm tangentially arranged. These occupy the space between the outer mesenchyme and the external limiting membrane of the spinal cord. Through the same region runs a vascular network, branches of which enter the nervous system accompanied by cellular elements. No fluid spaces could be identified with any certainty. It seems reasonable to conclude that the neural crest contributes very largely, if not entirely, to the formation of a thin investing membrane between the mesenchyme and the nervous system. Since blood vessels are included in it and since it lies between a relatively thick membranous sheet and the nervous system, it may be assumed to be a leptomeninx. This does not mean that mesenchymal elements are excluded from participation in the formation of the pia-arachnoid. It does indicate quite clearly, that in the absence of neural crest the pia-arachnoid fails to develop. Further experiments to determine the relative proportion of the neural crest and mesenchyme in the leptomeninx are in progress.

Flexner[3] made parallel experiments on Amblystoma and following Weed,[4] concluded that the presence or absence of neural crest produced no difference in the meningeal surroundings of the neural tube and that any difference which might result was due to the presence or absence of cerebral spinal fluid surrounding the nervous system. It is no part of our hypothesis that the cerebrospinal fluid is an unimportant factor in the differentiation of the meninges. Weed and his associates indicated quite clearly that the effusion of cerebrospinal fluid into the immediate surroundings of the nervous system is responsible for the delaminations occurring in the meninges. We may accept that as an established fact. But that fact does not preclude the possibility that the material which delaminates as a result of the activities of the cerebrospinal fluid is derived from other sources than mesenchyme. On the contrary, the experiments we have presented indicate that the neural crest and the mesenchyme aided by the presence of the cerebrospinal fluid form the investing membranes of the central nervous system.

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[3] Flexner, L. B., *Contributions to Embryology*, 1929, No. 110, Carnegie Inst., Wash., Pub. 394.

[4] Weed, L. H., *Contributions to Embryology*, 1917, No. 14, Carnegie Inst., Wash., Pub. 225.

5627

Action of Ilex Opaca on the Heart.

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Ilex opaca or American holly is an evergreen tree growing throughout the Atlantic section of the United States. The leaves and fruit are the parts of the plant that have been used in medicine; in domestic practice alterative properties have been assigned to it and by eclectic practitioners it has been used as an anti-intermittent, febrifuge, tonic and diaphoretic. The "Black Drink" by which the Indians "cleansed" themselves by drinking sufficient to cause vomiting was an aqueous extract prepared from the leaves of the holly. The dried leaves of certain species of ilex are used by the inhabitants of Paraguay to prepare a stimulating beverage.

The present experiments were undertaken to determine the effects of extracts of the above fruit on the amphibian heart. Male frogs *(Rana pipiens)* were used. A cannula was tied in the inferior vena cava and the heart arranged for perfusion by the Symes' method, with Ringer's solution in one bottle and Ringer's solution plus the drug in the other. The movements of the lever were recorded on a smoked drum. The extract was prepared by macerating the dried fruit in number 20 powder with 70% alcohol for 4 days and filtering. When required for use the alcohol was evaporated off and the thick syrupy remains added to frog Ringer's solution.

Perfusion in a proportion of one part of the crude drug in 750 parts of Ringer's solution resulted in an increase in the amplitude of the beat. Although the diastole of the heart was increased to some extent the main effect was a more complete shortening of the muscle. After allowing the heart to return to normal by perfusing with Ringer's solution only, the above effects of the drug could be demonstrated as often as desired. This increase in amplitude was accompanied by either a slight decrease or no change in the rate. When the concentration of the drug was increased to one in 300 there was a marked decrease in the relaxation of the ventricle and in a short time the heart was arrested in systole; there was also a decrease in the number of impulses sent out by the sinus so that the heart was slowed.

Pancoast[1] found the fruit of *Ilex opaca* to contain tannin, pectin, albumin and 2 crystalline principles and organic salts of potassium calcium and magnesium. Venable[2] extracted from the leaves of the Yopon *(Ilex cassene,* Linn.) a white crystalline substance which he believed to be caffeine. In some respects the tracings made in this work resemble those obtained by Heathcote[3] and by Roth[4] on perfusion of the frog's heart with the zanthine compounds. However, on close examination there are certain features such as the systolic standstill which suggest a digitalis-like action.

An attempt to isolate the principle responsible for the characteristic effects and a more extensive investigation of the pharmacological action of the drug is now being carried out.

5628

Effect of Corpus Luteum Extracts in Suppressing Ovarian Activity in the Rat.

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(Introduced by H. B. Lewis.)

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Recent studies in this laboratory have confirmed the results of Payne, Peenan and Cortland,[1] Haterius and Pfiffner,[2] and Macht, Stickels and co-workers[3] on the inhibition of normal ovarian activity by preparations of the corpus luteum.

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[1] Pancoast, D. P., *Am. J. Pharm.*, 1856, **28**, 314.

[2] Venable, E. P., *Am. J. Pharm.*, 1885, **57**, 390.

[3] Heathcote, R., *J. Pharmacol. Exp. Therap.*, 1920, **16**, 327.

[4] Roth, G. B., *J. Pharmacol. Exp. Therap.*, 1927, **30**, 321.

[1] Payne, Peenan and Cortland, *Am. J. Phys.*, 1928, **98**, 243.

[2] Haterius, H. O., and Pfiffner, J. J., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 818.

[3] Macht, D. I., and Stickels, A. E., *Am. J. Phys.*, 1929, **88**, 65.

The experiments herein reported were carried out on our laboratory strain of albino rats. The general plan was to take a series of mature animals and follow the oestrus cycle for a period of time to be certain that all animals were normal. The animals were then to be treated with corpus luteum extracts to determine any effect on the length of the cycle. In certain cases control extracts, prepared from the anterior lobe and thymus and containing approximately the same total solids, were also used.

Due to the well known fact that the length of the cycle is profoundly influenced by the general health and environment of the animal, all precautions were taken to keep the animals under uniform conditions during the course of the experiment. In each experiment a number of rats were used to determine the control cycles, but usually only about 50% of these showed a regular cycle and could be used in the experiment.

The corpus luteum extracts, as well as the control extracts of anterior pituitary and thymus, were prepared by acid alcohol extraction of either fresh glands or carefully desiccated glands with the subsequent conversion of the active material into an aqueous solution. No difference was noticed between the fresh glands and desiccated glands as the source of active material. All injections consisted of 1 cc. of sterile solution and were given intramuscularly.

The rats used in these experiments were from 3 to 4 months old and weighed from 150 to 175 gm. We found that in rats weighing much less than 125 gm. the cycles were too irregular to be used for this work. During the course of the experiment the animals, with very few exceptions, gained from 2 to 5 gm. in weight each week. This fact, along with the general physical appearance of the animals seemed to indicate no deleterious effect from the extracts.

TABLE I.—*Results.*

Exp.	No. of Rats	1 cc. Fresh Gland	No. Control Cycles	Aver. Cycle Days	No. Treated Cycles	Aver. Cycle Days
I	8	Corpus Luteum 6½ gm.	34	4.7	27	8.2
II	4	Anterior Lobe 4 gm.	13	4.8	12	5.2
III	6	Corpus Luteum 5½ gm.	20	4.4	14	8.2
IV	5	Thymus Solution, 5½ gm.	17	4.4	19	4.2

The results appearing in our first experiments seemed very conclusive since the control cycles were very uniform (over 90% being either 4 or 5 days), whereas the treated animals showed a marked reaction to the extract and showed cycles ranging as high as 13 days in length. In sharp contrast, the solutions prepared from anterior lobe and thymus and containing approximately the same total solids were apparently without effect on the normal cycle. On a repetition of the first experiments, using a more dilute extract of corpus luteum, essentially the same result was obtained.

Since all extracts contained approximately the same total solids and very little protein, it seems that the corpus luteum extracts did contain some factor which acted directly in inhibiting ovarian activity and the effect was not merely due to an impairment of the general bodily metabolism.

5629

A Conditioned Emetic Reflex in the Pigeon.

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In 1912 one of us administered a series of daily doses of yohimbine hydrochloride to several pairs of pigeons and doves which were kept in quite large cages. It was our custom to walk into a cage, catch the pair of birds, and then place the dry pellets of the drug into the upper throat with long, curve-tipped artery forceps. These pellets were readily swallowed but, unless restrained, many of the birds almost immediately regurgitated part or all of the contents of their crops. Several days after beginning this treatment it was observed that some of these birds began to vomit as soon as the person administering the drug entered their cage for this purpose—and *before* the bird was caught for dosage. Not all of this group of birds were seen to give this response, and complete records of the behavior were not obtained; those tests were made for a wholly different purpose and were not favorable to a study of the conditioned reflex. Recently we have repeated these tests on mature and immature ring doves, definitely attempting to provide *equal* externals and stimuli to the several birds, and also making complete records concerning the time and degree of exhibition of both the normal and the conditioned reflex in each of the birds tested. It is the purpose of this paper to report, without further analysis, the data thus obtained in this recent study. The classical studies of Pavlov,[1] Thorndike[2] and others, have provided a large body of knowledge within which the present item may have a value sufficient to make it worthy of record.

Emesis serves in both mature and immature doves and pigeons not only to rid the food-crop of objectionable material but it is also employed by the mature birds as the normal method of feeding their young. Moreover, males often combine a light regurgitation with the "billing" which immediately precedes copulation with their mates. In these particular animals emesis therefore involves motor responses which are easily induced, and which (at least in adults) are apparently induced by quite dissimilar stimuli. Hanzlik[3] has shown that pigeon-emesis may be used for estimating the potency of intravenously injected digitalis preparations.

Yohimbine has a bitter taste to humans; this, and also the sharpness or angularity of the tablet fragments used (a 5-grain tablet cut into 4 parts), may have contributed to the bird's employment of vomiting movements against its presence in throat or crop. Yohimbine may have produced nausea, though in no case was the first or second dose regurgitated during the 10 to 15 minutes of observation which followed its administration. It was almost certainly responsible for a watery diarrhea which plainly developed in several birds. This diarrhea and the undernourishment incident to the repeated regurgitation of food prevent a prolonged study of this reflex in normal animals.

The 12 quite tame doves used in our recent tests were placed in pairs in large cages which were entered through man-sized doors. For some days preceding the tests the person who was to administer the yohimbine did not enter these cages; during the tests he entered the cages only to administer the drug. While entering the cage the white pill-box was always held plainly in sight and its contents were rather noisily rattled.

One of the 6 mature birds vomited as we entered the door to give it its fourth dosage—thus it showed the conditioned emetic reflex after only 3 doses; another bird was thus conditioned by 5 doses; 2 by 6 doses; one by 13 doses; and one was not conditioned by the total of 14 doses. The 2 last-named very stubborn cases were given double the usual dose (1¼ grain) after the 8th day. Of the 6 immature doves, one showed similar emesis—or perhaps only partially successful vomiting movements—after 6 doses; four after 7 doses; and one after 8 doses.

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[1] Pavlov, I. P., Lectures on Conditioned Reflexes, New York, 1928.

[2] Thorndike, E. L., *Animal Intelligence*, New York, 1898.

[3] Hanzlik, P. J., *J. Pharm. and Exp. Ther.*, 1928, **35**, 383.

After its development, or first exhibition, the conditioned reflex was not invariably obtained on succeeding days from all of the birds. In one mature bird it was invariable during the 11 tests. In the mature birds which developed the reflex it was thus present in 63% of the later tests. On several days the immature birds were dosed twice daily; and the 16th to 20th doses (for 3 birds continued beyond the 15th dose) were of double strength. During this latter period only one bird continued to show the reflex before feeding, and only in a few cases was the normal reflex observed after feeding. Omitting this exceptional period of heavy feeding and unusual response, 4 of the immature birds showed the conditioned reflex to the following extent; in 8 of 9 tests; in 8 of 8 tests; in 2 of 8 tests; and again in 2 of 8 tests. One bird sickened early, showed the reflex once, and died 4 days later; another exhibited the conditioned reflex on the 8th day and failed to do so on 8 days thereafter.

Summary. A conditioned emetic reflex was usually, but not always, obtained in mature and immature doves and pigeons after 3 to 8 oral administrations of yohimbine hydrochloride. Individuals vary greatly in their normal emetic response to this drug, in their development of the conditioned emetic reflex, and in the constancy of its exhibition after it is developed. Though emesis is utilized normally to a far greater extent in mature than in immature birds our tests do not show that the conditioned emetic reflex is obtained in one age-group more readily than in the other.

5630

Serological Tests With the Blood of Cavia porcellus and Cavia rufescens.

K. LANDSTEINER.

From the Laboratories of the Rockefeller Institute for Medical Research, New York City.

In order to gain further information on the inheritance of serological characters in the hybridization of species,[1] an attempt has been made to find an instance of fertile species hybrids where a serological differentiation of the parent types would be possible. A material answering these requirements consists of the two sorts of cavies mentioned in the title, the cross of which has been studied extensively by Detlefsen.[2] As established by this author, the F_1 females are fertile when mated with either of the parent species.

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[1] Landsteiner, K., and Van der Scheer, J., *J. Immunol.*, 1924, **9**, 213, 221.

When the blood and serum of the two species were allowed to interact, no hemagglutination or hemolysis took place. It was not difficult, however, to obtain hemolytic sera by several intraperitoneal injections of 0.5 cc. washed blood of the wild Brazilian guinea pig into the common domestic guinea pig. An experiment with one of the most active immune sera gave the following results:

TABLE I.

2 drops immune serum, 1 drop 2½% suspension of washed blood; tests kept at 37°.

Blood of											Reading after
10 common guinea pigs	0	0	0	0	0	0	w	0	0	0	5 min.
	0	0	0	0	0	0	d	0	0	0	1 hr.
5 hybrids	w	w	st	w	c						5 min.
	st	st	a.c	st	c						1 hr.
10 wild Brazilian guinea pigs	a.c	c	a.c	a.c	c	c	c	c	a.c	c	5 min.
	c	c	c	c	c	c	c	c	c	c	1 hr.

w=weak; d=distinct; st=strong; a.c=almost complete; c=complete hemolysis.

In all, 20 Brazilian and 23 common guinea pigs were examined. Whereas the cells of all the former reacted positively with varying intensities, the blood of only one common guinea pig gave a distinctly positive reaction, although to a considerably lesser degree than any individual of the other species. The bloods of 6 hybrids (♂ *Cavia rufescens* × ♀ *Cavia porcellus*) showed positive reactions, but with one exception these were weaker than those obtained with the wild type.

On inactivating the sera, similar differences were demonstrable by hemagglutination.

It is proposed to extend these studies to further generations and to search for other reactions differentiating not only the blood cells but, if possible, also the proteins of the two kinds of animals.

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[2] Carnegie Institution of Washington, Publication No. 205, 1914.

5631

Observations on Serological Reactions with Albumose Preparations.

K. LANDSTEINER and J. VAN DER SCHEER.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

In continuation of the experiments briefly reported in this journal,[1] the following observations were made.

Immune sera prepared with azoheteroalbumose or azoprotoalbumose did not distinguish between the azocompounds of heteroalbumose and protoalbumose of the same origin. There was a distinct specificity, however, with some immune sera in tests with azoalbumoses derived from different material such as horse serum, eggwhite or Witte peptone. The reactions just mentioned were inhibited by azoalbumoses, particularly those of the same origin as the material used for immunization.

Since in most of the experiments described, crude preparations were used, the question arises whether the precipitin reactions with the azoalbumose preparations are due to the albumoses themselves or to the presence of other substances, such as metaprotein or plastein. Evidence against the latter assumption is the fact that reactions were also obtained with azocompounds of purified hetero- and protoalbumose* from Witte peptone and that the reactions with azometaprotein and azoplastein were rather weaker than those given by the azoalbumoses.

By immunization with a crude preparation of heteroalbumose (not coupled with diazobenzene) made by peptic digestion of horse serum, immune sera were obtained which gave precipitin reactions of moderate strength with the substance used for immunization and furthermore with metaprotein, prepared by short peptic digestion of horse serum, and with unchanged horse serum. No reactions took place with heteroalbumoses made from egg white or Witte peptone. On the other hand, in conformity with previous work,[2] a common anti-horse serum precipitin reacted only with horse serum but not at all with the metaprotein and the heteroalbumose from horse serum. The reactions of the immune serum

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[1] Landsteiner, K., and Van der Scheer, J., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 812.

* We are greatly indebted to Dr. P. A. Levene for supplying us with these preparations.

[2] Michaelis, L., *Deutsche Med. Wochenschr.*, 1904, **34**, 1240.

TABLE I.

Three drops of immune serum were added to 0.2 cc. of the antigen. The dilutions refer to 5% stock solutions.

Immune serum prepared by injection of azoheteroalbumose from	Reading after	Azoheteroalbumoses prepared from											
		Horse serum dilution 1:				Egg white dilution 1:				Witte peptone (purified preparation) dilution 1:			
		100	500	2500	12500	100	500	2500	12500	100	500	2500	12500
	hours												
Horse serum	3	++±	++	±	0	0	ftr	0	0	±	+	ftr	0
Witte peptone	3	tr	±	ftr	0	0	tr	ftr	0	+	++	±	0

produced by the injection of the albumose could be inhibited by addition of a sufficient amount of a solution of the preparations of hetero-, proto-, or deuteroalbumose from horse serum, whereas albumoses of other origin, used in the same concentration, had no appreciable effect.

5632

On the Resistance and Filterability of the Agent Transmitting Leucosis.*

J. FURTH. (With the assistance of Charles Breedis.)

From the Henry Phipps Institute, University of Pennsylvania.

Blood cells preserved in 50% glycerin for 51 days produced leucosis in 3 of 4 injected fowls, after an incubation period of from 51 to 56 days. The same material, kept in glycerin for 104 days, produced leucosis in 3 of 4 fowls injected after a period of incubation from 35 to 82 days. The fresh blood employed in these tests caused leucosis in all 5 inoculated birds 12 to 45 days after injection. The fresh blood, as well as the blood preserved in glycerin, was injected in amounts of 0.5 cc.

Attempts to determine whether the agent of leucosis resists desiccation have repeatedly failed until recently fowls were injected with blood, dried rapidly *in vacuo* over sulphuric acid, at a temperature of about +4°C. and kept at this temperature for 15 days. All 3 injected fowls developed leucosis after 34 to 45 days. Likewise successful was the inoculation of blood dried over calcium chloride under similar conditions. Of 3 injected fowls 2 developed leucosis. The same fresh blood caused leucosis in 2 injected fowls, after a period of incubation of 13 to 19 days.

Previous failures to preserve this agent under similar conditions may have been due to the relatively mild activity of the agent, now increased considerably. This "increase in virulence" is manifested by the very high percentage of successful inoculations, decrease in the incubation period, and associated increase in the concentration of the transmissible principle in the blood stream as determined by titration.

Leucemic blood, kept in the refrigerator at +4°C., was still active after 7 days, but had lost its activity after 16 days.

Filtration has been much facilitated by passing blood through a coarse filter before passage through a fine filter. Plasma passed through a fine Berkefeld (W) filter after preceding filtration through a coarse filter caused leucosis in 2 of the 4 fowls inoculated. The same unfiltered plasma caused leucosis in 2 of the 3 injected fowls.

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* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases.

5633

Relation of Age and Breed to Susceptibility in Leucosis of Fowls.*

E. L. STUBBS AND J. FURTH.

From the Veterinary School and The Henry Phipps Institute of the University of Pennsylvania.

The transmissible avian tumors discovered by Rous and his associates are not transferable to birds other than fowls. The similar tumor of Fujinami, however, has been successfully transmitted to ducklings (Fujinami, Gye). The infectious diseases of fowls caused by a filterable virus, such as fowl pest and fowl pox, are readily transmissible to several species of birds. Leucosis of fowls is, according to Ellermann and Bang, not transmissible to guinea fowls, doves and turkeys.

An investigation was undertaken to determine the relative susceptibility of various breeds of fowls to transmissible leucosis and the possibility of transmitting leucosis to birds other than fowls. It was first necessary to determine the influence of age on susceptibility to transmissible leucosis.

Three groups of Barred Rock chickens, each containing 6 members, were injected with 0.6 cc. blood of a leucemic fowl. The fowls of one group were about 4 months of age, of the second about one year and of the third over 2 years of age. The result of these inoculations is shown in Table I.

TABLE I.

Approximate age of fowls	No. injected	No. of inoculations		Average period incubation	Successful inoculations	
		Successful	Unsuccessful		Recovered	Died
				days		
4 months	6	4	2	22	0	4
1 year	6	4	2	46	2	2
Over 2 years	6	2	4	48	1	1

This table indicates that older birds are less susceptible to transmissible leucosis than younger birds and that the period of incubation is shorter in the younger birds.

All the breeds of the domestic fowls that were tested were susceptible to transmissible leucosis. The fowls used for these tests

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* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases.

were 4 to 5 months old and were inoculated intravenously with 0.6 cc. of leucemic blood. Of 6 Barred Rocks, 4 acquired leucosis; of 6 White Leghorns, 4; of 6 Rhode Island Reds, 2; of 6 mixed breed, 2. In another series of inoculations of 6 Bantams, all acquired leucosis.

Of the gallinaceous birds, other than domestic fowls, the Guinea fowl was tested and all of the 10 young Guinea fowls that were injected resisted the inoculation. Likewise unsuccessful were the attempts to transmit leucosis to 12 pigeons.

5634

Fate of Leucemic Blood of Fowls After Transfusion.*

R. P. CRANK AND J. FURTH.. (With the assistance of Ruth Klingelhofer.)

From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

Leucemic blood was transferred into healthy animals and the fate of the immature leucocytes was followed in the circulating blood and in the blood forming organs of the recipient. Such a study might reveal whether leucemia is the result of multiplication of the transfused immature leucocytes in a susceptible host. In addition, the fate of the transmissible agent in the blood of the recipient was studied because it was assumed that if leucosis were secondary to infection, the infective agent might be recovered from fowls that exhibited no leucemic blood changes.

The fate of transfused leucemic cells. Six young chickens were transfused with 20 to 35 cc. of the blood of fowls with severe myeloid leucemia, after 13 to 35 cc. of blood had been removed from the circulation of each recipient. Following transfusion, there was an immediate increase of the leucocyte count. Leucocyte counts of the blood of donors and recipients, mixed *in vitro* in proportions estimated to be those in the recipients, gave values from 2 to 3 times as high as those found in the recipients 30 minutes and 1 hour after transfusion. Figures for red cells and hemoglobin in the mixtures, on the other hand, agreed roughly with the figures found in the transfused animal. This observation indicated that leucocytes had been removed from the peripheral circulation. There was a slight further fall in the leucocyte counts during periods

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* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases.

varying from 1 to 2 days, which was followed by a slight rise during a period of about one day. Subsequently the leucocyte count of 4 of the 6 transfused chickens fell to normal within a few days with a complete disappearance of the immature cells. Three of these 4 birds showed no further changes, but one developed erythroleucosis 47 days after the transfusion.

In 2 of the transfused chickens there was, instead of a drop, a rapid rise in the number of the circulating leucocytes, and both birds died in 3 days with the blood picture of leucemia. In one, the leucocyte count before transfusion was 27,500, one half hour after transfusion, 365,000, and in 3 days, 910,000.

This secondary elevation was much higher than could be attributed to the mechanical effect of transfusion. The large number of mitoses in the circulating blood gives further evidence that active multiplication of the transfused leucemic blood cells had occurred in the body of the host. This opinion was strengthened by microscopic examination of the organs. There was engorgement of the blood vessels with primitive large mononuclear leucocytes, and many of these cells were undergoing mitotic division. The bone marrow contained abundant fat and showed only a slight degree of hyperplasia. There were small foci in the liver resembling myeloid metaplasia. The changes observed are best explained by assuming that the host acted as an incubator for the leucemic blood introduced into its circulation.

Fate of the transmissible agent. Five young chickens were injected intravenously each with 0.5 cc. of the blood of a fowl with severe myeloid leucemia. Blood was removed from the veins of 3 of these chickens 30 minutes, 1, 7, and 15 days after inoculation and injected immediately into the veins of normal chickens in amounts of 0.5 cc. Fifteen days after inoculation, only one of them remained normal. Each of 4 normal chickens were injected with 0.5 cc. of blood, from this apparently healthy fowl. Each of 5 fowls were inoculated with 0.7 cc. blood of an apparently healthy fowl transfused 30 days previously with 20 cc. leucemic blood.

These experiments have shown that the transmissible agent is demonstrable in the blood stream of the recipient 30 minutes after inoculation. Most, if not all of it, leaves the circulation within 24 hours and reappears within 7 to 15 days in fowls that develop leucosis. In fowls that remained free from leucosis, the transmissible agent was not found in the circulating blood.

Previous observations[1] gave evidence that avian leucosis can be produced by cell-free material. The present study indicates that the leucemic cell itself is capable of autonomous growth.

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[1] Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 449.

Rous[2] observed that tumor cells and their filtrates behave differently when brought in contact with immune sera, the former being neutralizable, the latter not. Similar observations were made by Sittenfield, Johnson and Jobling.[3] It may be assumed that this difference is due to the position of the agent, intracellular in one instance or free in the other. Our observations suggest the occurrence of 2 distinct processes: (a) a filterable agent causes neoplastic growth and (b) its product, the neoplastic cells are capable of autonomous growth.

5635

Effect of Suprarenal Cortical Extract on Nitrogen and Sugar Elimination in Depancreatized Dogs.

ALFRED E. KOEHLER.

From the Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barbara, California.

In previous publications we[1] described a new calorigenic principle obtained from the suprarenal cortex or whole gland. The active substance is combined in the lipoid fraction and is extracted from the tissue with ethyl alcohol. After evaporation of the alcohol and extraction of the residue with benzene or ether, the active lipoid protein-free fraction is obtained. The water-soluble fraction of the lipoid contains epinephrin besides the active substance.[2] Epinephrin, however, is less firmly bound by the lipoid and can first be removed by prolonged washing or mild acid hydrolysis in the absence of oxygen. The active principle is unstable, but in the absence of oxygen we have kept a preparation active for 5½ months. In our earlier work the extract was administered orally but effects were not always constant. Given hypodermically, approximately one-tenth to one-fifteenth the amount is required and the results have been much more consistent.

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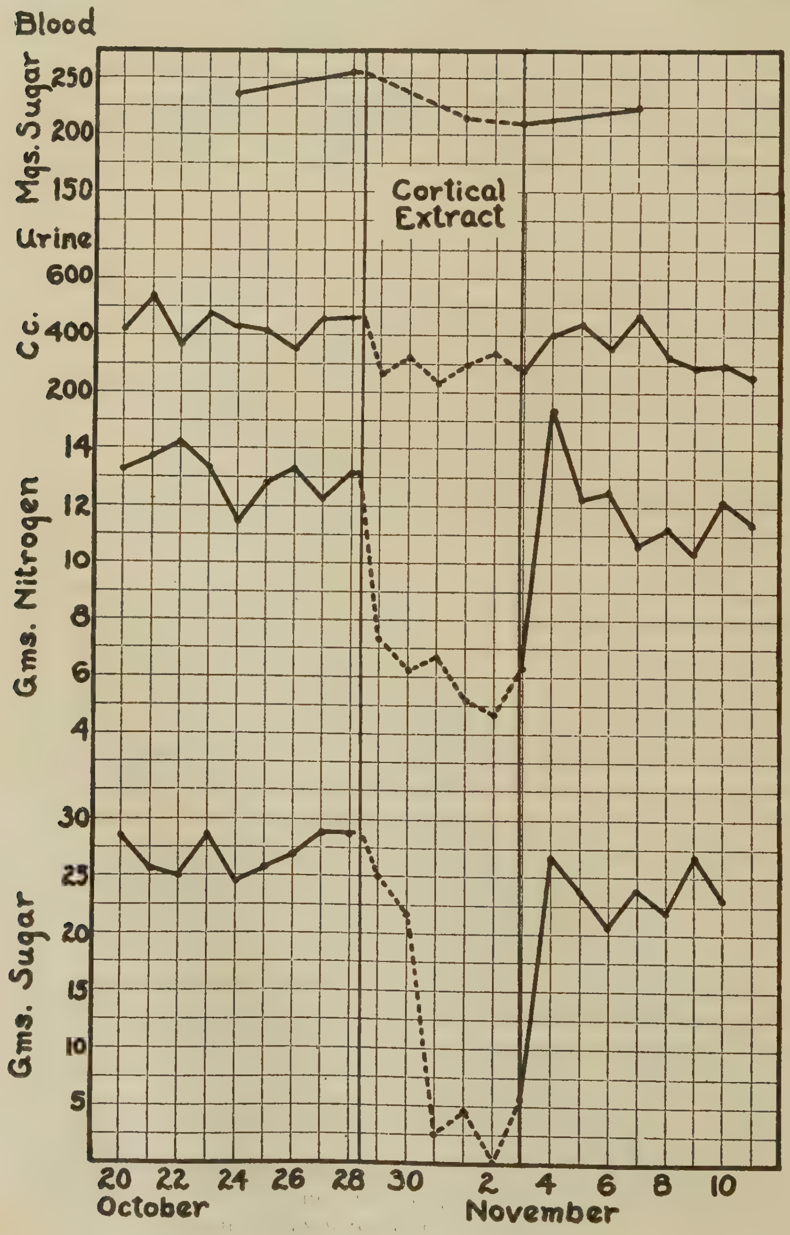
[2] Rous, P., *J. Exp. Med.*, 1913, **18**, 416.

[3] Sittenfield, M. J., Johnson, B. A., and Jobling, J. W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 517.

[1] Koehler, A. E., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 296. Koehler, A. E., and Eichelberger, L., *Am. J. Physiol.*, 1929, **90**, 2.

[2] Koehler, A. E., and Eichelberger, L., *J. Biol. Chem.*, 1930, **87**, 38.

Besides the calorigenic effect, the preparation has been found capable of increasing the mechanical efficiency in certain cases of abnormal muscular fatigue as *myasthenia gravis* and Addison's disease. In such cases the abnormal creatine excretion also disap-



peared. Since these effects could possibly be explained by an increase in carbohydrate metabolism, we have studied the effect of the calorigenic principle upon the sugar and protein metabolism in depancreatized dogs.

The accompanying chart shows a typical experiment in a dog 3 months after operation. During this period the dog was placed on exactly 1,000 gm. lean meat plus pancreatin and 14 units insulin, which permitted a sugar elimination of 25 to 30 gm. daily. One cc. of cortical extract representing 45 gm. of whole gland given hypodermically daily caused a diminution in the urinary sugar and nitrogen. No constant ratio was noted between sugar and nitrogen elimiination. In several experiments the decrease in the sugar elimination was greater than that which could be accounted for on the basis of decreased protein metabolism.

The decrease in the sugar elimination was constant in 8 experiments on 3 depancreatized dogs when a calorigenic active substance was given. In 4 of these experiments the extract was given by mouth and in 4 it was injected hypodermically. In the first 2 dogs the treatment periods were limited to 3 or 4 days so as to observe the return of the sugar and nitrogen to the level before treatment. In experiments on the third dog attempts were made to extend the treatment over longer periods. However, after 4 to 6 days of treatment the sugar elimination gradually rose again in spite of continuous treatment. A period of a week or more had to elapse before the urinary sugar could again be depressed. Death of the first 2 dogs has prevented our repeating the prolonged treatment effect on them.

Control experiments with epinephrin (0.25 to 1.0 mgm. hypodermically or 10 to 30 mgm. orally) have failed to give any depression of the sugar eliminated. Epinephrin given hypodermically elevated the sugar elimination in all cases.

5636

A Study Concerning the Incidence of Streptococcus Epidemicus.

C. W. BONYNGE. (Introduced by John F. Kessel.)

From the Department of Experimental Medicine, University of Southern California, and the Laboratory of the Los Angeles Certified Milk Commission.

The occurrence of septic sore throat and of carriers of *Streptococcus epidemicus* during inter-epidemic periods has become a matter of importance. Pilot and Davis[1] have recently published results of a study of this subject in Chicago.

It is of interest to note that an epidemic of septic sore throat has never been experienced in Southern California. In so far as can be determined, bacteriological studies for the identification of *S. epidemicus* have been neglected.

Our investigations over a period of 4 months, have resulted in rather interesting findings. In a group of 250 employees of a local institution, 7 positive cultures of *S. epidemicus* were obtained from throat swabs. Each individual harboring *S. epidemicus* was in good health and gave no history of having had sore throat of the epidemic type, but in each instance presented some degree of tonsillar hypertrophy. This represents a carrier group of 2.8%.

In a second group of 66 individuals presenting themselves for operation for hypertrophied tonsils, 6 positive cultures were found. A history of previous attacks of probable septic sore throat was not obtainable. Of these individuals 9% harbored *S. epidemicus.*

Pilot and Davis have stated that a higher per cent of positives is obtained when cultures are made from depths of tonsil crypts. Our experience confirms this observation.

A positive culture was obtained from a typical clinical case of septic sore throat with marked edema of pharynx and glottis. This case recovered without secondary complications and was the only person in a family of 4 to be stricken.

Two cases of otitis media in children with subsequent mastoiditis were found to be of *S. epidemicus* origin.

Among 5 cases of septicemia, all with positive blood cultures, in a hospital for children, 2 proved to be due to *S. epidemicus.* In neither case could a history of previous typical septic sore throat be elicited. One of these cases had an acute endocarditis which was verified at autopsy.

The initial specimens in all cases were obtained in nutrient broth, and cultural studies carried out on 20% ascitic blood agar as advised by Pilot, Hallam and Davis.[2]

Conclusion. Over a period of 4 months, 18 strains of *Streptococcus epidemicus* have been recovered; 5 from individuals suffering from acute infections and 13 from human carriers. Thus, verification of the presence of *S. epidemicus* in various types of infection and in the carrier state during inter-epidemic periods is offered.

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[1] Pilot, Hallam and Davis, *J. Infect. Dis.*, 1930, **47**, 503.

[2] Pilot, Hallam and Davis, *J. Am. Med. Assn.*, 1930, **95**, 264.

5637

A Pharmacological Study of the Inhibitory Mesenteric Nerves to the Intestine.*

MAX D. HENDRICKS AND C. H. THIENES.

From the Departments of Physiology and Pharmacology, University of Southern California, Los Angeles, California.

An attempt to understand better some of the actions of cocaine, ephedrine and other drugs on the intestine, led to the use of the excised nerve-muscle preparation of the intestine described by Thomas.[1] At the time our experiments were begun, McSwiney and Robson[2] had reported the use of a similar preparation of the stomach, and since then there have appeared papers by Finkelmann[3] and McSwiney and Robson,[4] describing results with the nerve-muscle preparation of the intestine. Our results in general confirm those of the authors mentioned, and extend the study further.

A modified Locke solution was used to immerse the tissues, which were removed as required from rabbits and dogs anesthetized with urethane and chloretone or with amytal. Records were made of the movements of only the longitudinal muscle fibres. Stimulation of the mesenteric nerves was by means of the Harvard inductorium, through platinum electrodes applied to the periarterial nerves. Slowly repeated shocks (1 to 10 per second), as well as rapid shocks (12 to 60 per second), and minimal effective as well as supermaximal strength, stimulating for periods up to 1 minute, resulted only in decrease in tone and inhibition of the movements of the intestinal muscle. The inhibition persisted for several seconds after stimulation ceased, and in a few instances, there was over-recovery of tone, with gradual return to normal, thus confirming the results of Thomas[1] All effects of nerve stimulation were closely imitated by epinephrine on each individual preparation. In no instance did a primary augmentation of intestinal activity result from nerve stimulation.

The inhibition was not prevented by nicotine or atropine in 1:10,000 concentration, or by a mixture of nicotine and atropine, each 1:10,000. Ergotamine tartrate or ergotamine methane sul-

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* This research has been supported by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Thomas, J. E., *Am. J. Physiol.*, 1926, **76**, 228.

[2] McSwiney, B. A., and Robson, J. M., *J. Physiol.*, 1929, **68**, 124.

[3] Finkelmann, B., *J. Physiol.*, 1930, **70**, 145.

[4] McSwiney, B. A., and Robson, J. M., *J. Physiol.*, 1931, **71**, 194.

phonate† 1:150,000 to 1:75,000, ephedrine-HCl 1:20,000 to 1:10,000, cocaine-HCl 1:75,000, nicotine 1:1,000, atropine 1:1,000 and a mixture of nicotine and atropine, 1:1,000 each, markedly diminished or entirely abolished the inhibitory effect on intestinal movements of electrical stimulation of the mesenteric nerves.

5638

An Analysis of the Actions of Cocaine on Excised Smooth Muscles.*

LILLIAN SHUTTER AND C. H. THIENES.

From the Department of Physiology and Pharmacology, University of Southern California, Los Angeles, Calif.

In a previous report[1] there was presented evidence that cocaine is not a general sympathomimetic drug. Since most of the studies of the action of this drug on the iris (Gold, Miller, Koppanyi, Yonkmann[2]) indicate an action on the terminal mechanism of the sympathetic nerves to this organ, a further analysis of the action of cocaine on other organs was carried out. All studies were on excised tissues bathed in Tyrode solution or modified Locke solution. The tissues were taken from animals killed by a blow, or under urethane and chloretone or amytal anesthesia. Only experiments on small intestine, colon and uterus of the rabbit are described in this report.

Small intestine. Five segments of small intestine of the rabbit were shown to respond to 1:10,000 cocaine HCl by contraction. This action was not prevented by previous treatment of the tissue with nicotine and atropine, 1:50,000 each. Of 15 segments which were depressed by cocaine 1:10,000, 5 were depressed by cocaine after nicotine and atropine 1:20,000 each, 5 were depressed by cocaine, but not by epinephrine, after ephedrine 1:10,000, and 5 were depressed by cocaine, but not by epinephrine, after ergotamine, 1:75,000. These concentrations of ephedrine and ergotamine were shown to prevent the inhibitory effect of electrical stimulation

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† The authors are indebted to the Sandoz Chemical Works, Inc., for a generous supply of ergotamine tartrate and ergotamine methane sulphonate.

* This research has been supported in part by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Thienes, C. H., *J. Pharm. Exp. Therap.*, 1928, **33**, 21.

[2] Gold, H., *J. Pharm. Exp. Therap.*, 1924, **23**, 365; Miller, G. H., *Ibid.*, 1926, **28**, 219; Koppanyi, T., *Ibid.*, 1930, **38**, 113; Yonkman, F. F., *Ibid.*, 1930, **40**, 195.

of the mesenteric nerve, yet cocaine depressed several such poisoned muscles.

Colon. Five segments of rabbit colon were contracted by cocaine, 1:10,000. This action of cocaine was in no way influenced by nicotine or atropine, 1:20,000.

Uterus. Four segments of excised uterus of the rabbit were contracted by cocaine, 1:10,000 both before and after ergotamine reversal of epinephrine action.†

Conclusions. Since the drugs which are known to paralyze ganglia and nerve terminations of the autonomic nervous system failed to prevent either the augmentor or depressant effects of cocaine on the small intestine, colon and uterus of the rabbit, it seems reasonable to conclude that this alkaloid acts on the muscle, rather than the nerves of the organs studied.

5639

Studies on Cyanide-Stable Respiration.

JOHN FIELD, 2nd, AND SALLY M. FIELD. (Introduced by E. G. Martin.)

From the Department of Physiology, Stanford University.

The opinion has long been held that the respiration of animal tissues is completely inhibited by cyanides.[1] However, Dixon and Elliott[2] have shown that in most tissues there is an appreciable oxygen consumption in the presence of the cyanide radical.

We have found that the cyanide-stable fraction of the respiration of the liver of the frog, *Rana pipiens,* is quite appreciable. The frogs were pithed and the liver promptly removed. The tissue was then minced with a razor moistened with Ringer's solution into slices 0.5 mm. or less in thickness, under which conditions Warburg[3] has shown that oxygen and cyanide have free access to the several cells. The minced liver, suspended in phosphate Ringer's,[4] was placed in the respiration chamber of a Warburg manometer. A cup containing 7% KOH removed the CO_2 produced. All the manometers were calibrated according to the method of Warburg.[5] The respiration chamber was placed in a water thermostat at 21.5°C., and shaken 70 times per minute.

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† The authors are indebted to the Sandoz Chemical Works, Inc., for a generous supply of ergotamine tartrate and ergotamine methane sulphonate.

[1] Macleod, J. J. R., "Physiology and Biochemistry in Modern Medicine." 1930, 622.

[2] Dixon, M., and Elliott, K. A. C., *Biochem. J.*, 1929, **23**, 872.

[3] Warburg, O., *Biochem. Z.*, 1923, **142**, 317.

[4] Dickens, F., and Simer, F., *Biochem. J.*, 1930, **24**, 1301.

The cyanide-stable fraction of the respiration was measured by placing a weighed portion of a given liver prepared as described in each of 2 respiration chambers, so that all comparisons were made between portions of one liver. One of the chambers contained phosphate-Ringer's, the other phosphate-Ringer's which was M/210 with respect to KCN. Dixon and Elliott[2] have shown that M/300 KCN produces maximal inhibition in most tissues, and we have found no appreciable difference between the effects of M/210 and M/21 KCN. In every case the cyanide was neutralized.[2]

The oxygen consumption of a given suspension of tissue was determined over a period of at least 2 hours, after previous soaking in the solutions for 15 to 45 minutes. Only those experiments in which the oxygen consumption is approximately linear with respect to time are reported. The ratio $\frac{\text{cyanide-stable respiration}}{\text{total respiration}} \times 100$ in 8 experiments varied between 15.62 and 57.9%, average 35.3.

Urethane is known to interfere with cytochrome reduction. If this is quantitative, one might assess the importance of the cytochrome b system in the cyanide-stable moiety of the respiration by treating with both cyanide and urethane. Liver tissue, prepared as before was divided into 2 portions, which were suspended in phosphate-Ringer's (a) including M/210 KCN; (b) M/210 KCN and 5% ethyl urethane. The $\frac{\text{Respiration in cyanide + urethane}}{\text{Respiration in cyanide}} \times 100$ was 71.0, 61.9, 71.2, 59.5, 39.0% respectively.

It is clear that a portion of the cyanide-stable respiration is not inhibited by ethyl urethane, and it is possible that we are here dealing with a new type of respiratory system. Further experiments on the properties of cyanide-stable and cyanide-urethane-stable respiration are under way in this laboratory.

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[5] Warburg, O., "*Uber den Stoffwechsel der Tumoren,*" 1926.

5640

Cyclic Variations in Pulse Rate in Women and Their Relationship to the Menstrual Cycle.

LILLIAN M. MOORE AND LULU MARIE JENKINS.

From the Division of Physiology, University of California Medical School, Berkeley.

The daily pulse rates of 7 women observed over periods of 2 to 13 months vary with each phase of the menstrual cycle and in no case show a static norm.

The pulse rate progressively increases during the phase of the cycle representative of the greatest activity (glandular and otherwise) of the genital tract; *i. e.,* from the end of the 1st to the end of the 3rd week of the menstrual cycle. It progressively decreases during the following 2 weeks of lessened activity, the lowest point being reached near the end of or after the completion of menstruation.

The rhythm in pulse rate shows a significant resemblance to the rhythm in the primary events of the menstrual cycle.

The rhythm can be statistically shown: (1) by a simple smoothing process by which daily fluctuations are eliminated if the readings are continuous, or by a combined compositing and smoothing method if discontinuous; (2) by a method which gives the significance of the difference between the low and the high values in the rhythm.

The above is a summary of the material described in a manuscript completed by Professor Moore before her death. In order to make the results of these investigations available the manuscript, with charts, has been placed on file in the University of California Library, Berkeley. A copy of the manuscript will be loaned upon application to the library.

5641

Effects of Heteroplastic Transplantation of the Ear in Amblystoma.*

DOROTHY RICHARDSON. (Introduced by Ross G. Harrison.)

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When the otic plate is exchanged between early embryos of *Amblystoma punctatum* and *A. tigrinum*, the results agree essentially with similar transplantations of the limb, eye, and nasal placode, but are modified due to the location of the ear in the head. (Harrison,[1] Twitty,[2, 3] Schwind,[3] Stone,[4] Burr.[5])

The grafted auditory vesicle develops normally and becomes functional in both species, judging by the equilibratory reactions. The relative size of the normal and transplanted ears was estimated by weighing paper models of the labyrinth. The weights indicate that the tigrinum graft on the punctatum host, though larger than the normal punctatum ear, becomes greatly retarded in its growth, as compared with its own species control, while the punctatum ear on tigrinum, though smaller than the normal tigrinum, exceeds the normal punctatum ear in size.

The cartilaginous capsule, measured in the same way, shows some response to the presence of the transplant, but the capsule around the tigrinum graft is proportionately smaller than that surrounding the normal tigrinum ear. Conversely, the capsule around the punctatum graft on tigrinum is larger than the normal capsule in the punctatum donor. This may possibly be due to a difference in the amount of cartilage-forming material available in the 2 species. None of the other adjacent cartilages is affected, with the exception of the columella, which shows corresponding deviations in size.

Models of the muscles which originate on the ear capsule demonstrate no growth responses, but their position on the larger or

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* A part of this work was done with the generous aid of the Margaret E. Maltby Fellowship from the American Association of University Women, 1929-1930.

[1] Harrison, R. G., *Proc. Nat. Acad. Sci.*, 1924, **10**, 69; *Anat. Rec.*, 1925, **31**, 299; *Proc. X Internat. Cong.*, Budapest, 1929, 642; *Arch. f. Entwmech.*, 1929, **120**, 1.

[2] Twitty, V. C., *Diss.*, Yale Univ., 1929; *J. Exp. Zool.*, 1930, **55**, 43.

[3] Twitty, V. C., and Schwind, J. L., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 686.

[4] Stone, L. S., *J. Exp. Zool.*, 1930, **55**, 193.

[5] Burr, H. S., *J. Exp. Zool.*, 1930, **55**, 171.

smaller capsules is shifted. In Twitty's study of the eye muscles after heteroplastic transplantation of the eye, hypertrophy or atrophy was marked.[2]

The studies of Detwiler,[6] Burr,[7] Twitty,[2] Schwind,[3] and others have established the fact that hyperplasia in nerve centers occurs when the peripheral sensory load is increased, and that hypoplasia results when it is diminished. Cell counts of the acoustico-facial ganglion, which probably arises from the graft, show it to be larger on the side of the tigrinum transplant, and smaller with the reciprocal graft from punctatum. In the gray matter of the area acoustica of the medulla in the punctatum host, there is a 15 to 20% increase in the number of cells on the side of the operation, and a decrease of approximately 10% in the corresponding region on tigrinum.

Mauthner's cell is not obviously altered as to the arrangement of its dendrites by the entrance of a larger or smaller nerve. No response can be detected in the cerebellum, to which a few of the eighth nerve fibres probably run.[8, 9] The effects produced in the area acoustica, however, are in agreement with conclusions from morphological studies that it is the chief center in the brain of urodeles for the endings of the acoustico-lateralis system of nerves.[9]

5642

A Method for Demonstrating Prepituitary Maturity Hormone in the Blood of Non-Pregnant Women.

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Since the prepituitary maturity hormone has been shown to produce puberty, follicle ripening and corpus luteum formation[1, 2] and since this hormone has been demonstrated in the blood serum and urine of pregnant women and female castrates,[2, 3, 4] it appeared logical to expect its presence in the blood of women during the menstrual cycle. Aschheim and Zondek[2] have determined 3 types of action exerted by the maturity factor, follicle ripening (A P R I), blood spots (A P R II) and corpus luteum formation (A P R III). They ascribe these results to 2 distinct hormones which they call Prolan A producing A P R I, and Prolan B causing A P R II and III, but have been unable to devise an exact method for separating these factors.

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[6] Detwiler, S. R., *Proc. Nat. Acad. Sci.*, 1920, **6**, 96; *J. Exp. Zool.*, 1926, **45**, 399; *Quar. Rev. Biol.*, 1926, **1**, 61.

[7] Burr, H. S., *J. Exp. Zool.*, 1916, **20**, 27; *J. Comp. Neur.*, 1924, **37**, 455.

[8] Larsell, O., *J. Comp. Neur.*, 1920, **31**, 259.

[9] Herrick, C. J., *J. Comp. Neur.*, 1914, **24**, 343.

[1] Smith, P. E., *Am. J. Physiol.*, 1927, **80**, 114.

[2] Zondek, B., and Aschheim, S., *Arch. f. Gynäk.*, 1927, **130**, 1.

[3] Aschheim, S., and Zondek, B., *Klin. Wchnschr.*, 1928, **7**, 1401.

[4] Fluhmann, C. F., *J. Am. Med. Assn.*, 1929, **93**, 672.

We have attempted to devise a method for demonstrating the presence of either or both Prolan A and B in the blood of non-pregnant women. A convenient material for control is supplied by the serum of pregnant women of which 0.2-0.5 cc. contains a M. U. or R. U. The untreated serum could therefore be used and equivalent amounts of the extracts of the same serum compared with this.

The following technique was employed: a. Blood serum obtained from women at various phases of the cycle, from 1 to 22 cc. in amount, was injected into immature mice and rats without conclusive results. b. The proteins of the blood serum were precipitated with acid alcohol (one part serum, 3 parts 95% alcohol). After dilution to 50% the supernatant was separated by centrifugalization and concentrated to a volume of 6-8 cc. by means of an air current at room temperature. No physiological effect was noted although pregnancy blood similarly treated and used as a control showed that the extraction method was effective, with minimal loss of potency. The method finally adopted, by which the presence of the A P R I reaction was demonstrated, is as follows: 40 cc. of blood is rubbed with 30 gm. of anhydrous Na_2SO_4, powdered and extracted thrice in a separating funnel with ether (each time 100 cc.). The ethereal extract may be used for our female sex hormone test.[5] The residual sludge is dried by evaporation, repowdered and shaken for 10 minutes with 200 cc. of 60% ethyl alcohol. The mixture is centrifuged and the supernatant fluid placed in an evaporating dish before an electric fan. When the volume has been reduced to approximately half, a scum forms, which is removed by recentrifugalization. The residual dry substance finally obtained by complete evaporation is dissolved in 6 cc. of water and injected into immature rats.

Sixty per cent alcohol was chosen because only small amounts of Na_2SO_4 are dissolved in it and yet the hormone is fully soluble at this concentration.

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[5] Frank, R. T., and Goldberger, M. A., *J. Am. Med. Assn.*, 1926, **87**, 1719.

The total extract is injected in 6 doses over a period of 3 days. The rats are killed 100 hours after the first injection. Their ovaries are cut in serial section.

Results. 1. A P R I is noted throughout the cycle to a varying degree. 2. The reaction appears strongest from 6 to 9 days from the onset of the preceding menstruation. 3. From the 6th to 9th day an indication of lutein change is noted. It is too faint to be interpreted as a full A P R III. 4. These results signify that approximately 25 M. U. L. is the maximum amount of Prolan A occurring in the circulation of the non-pregnant woman.

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Calcification of Tubercles by Administration of Calcium Chloride.

VALY MENKIN.

From the Department of Pathology, Harvard Medical School.

In previous communications[1, 2] it was shown that a vital dye, trypan blue, or a metal in the form of its salt, ferric chloride, when injected into the circulating blood stream rapidly accumulates in an area of inflammation, where the substance is fixed and fails to drain to the tributary lymph nodes. Subsequently it was shown in infected rabbits[3, 4] that repeated daily intravenous injections of ferric chloride are followed by an accumulation of iron in the caseous center of tuberculous areas.

The object of this study was to determine whether calcium would likewise penetrate from the blood stream into tuberculous foci. Such evidence might offer a reasonable explanation for the origin of this element in calcified tubercles. The literature fails to reveal any agreement on the effect of calcium in experimental tuberculosis. Michelazzi[5] found calcification of lesions in tuberculous rabbits treated with calcium. Hoyle,[6] who maintained that these earlier experiments were unsatisfactory, found no appreciable difference in the

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[1] Menkin, V., *J. Exp. Med.*, 1929, **50**, 171.

[2] Menkin, V., *J. Exp. Med.*, 1930, **51**, 879.

[3] Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1020.

[4] Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1931, **53**, 919.

[5] Michelazzi, *Gaz. d. Ospedali*, Milano, 1904, **25**, 425; abst. *Rev. de la Tuberc.*, 1904, **1**, 421.

[6] Hoyle, J. C., *Quart. J. Med.*, 1929, **22**, 451.

course of the disease between experimental and control groups. He, however, failed to make histological comparisons. Maver and Wells[7] found in guinea pigs that the amount of calcium in tuberculous lesions is not appreciably modified by adding calcium lactate to the usual diet. Spies[8] recently demonstrated that repeated administration of irradiated ergosterol causes calcification of tubercles.

In our experiments bovine tuberculosis was induced in rabbits by the intravenous injection of 0.01 mg. of a saline suspension of Ravenel strain. From 3 to 4 weeks later several of the rabbits were given daily intravenous injections of 5% $CaCl_2$ solution. On the first day each animal received 3 cc. of this solution; 4 to 6 cc. was injected on each of the subsequent days. After a variable number of injections these rabbits were killed. The lungs revealed extensive caseous tuberculosis. Some of the caseating areas appeared firmer to touch than usual. The tissues were fixed in 10% formaldehyde. Histological preparations were stained with hematoxylin and eosin. In addition special stains for calcium were made by the silver method of Von Kossa and by 1% aqueous sodium alizarin sulphonate. The latter test was performed in view of studies of Cameron,[9] who maintains that alizarin is the most reliable test for freshly deposited calcium in tissues. In all the microscopic sections there was complete agreement in the results as obtained by the 3 methods of staining for calcium. To serve as controls several tuberculous animals that received no $CaCl_2$ were killed at about the same time as the experimental rabbits. The lungs which revealed about the same extent of caseating tuberculosis as in the injected rabbits were studied histologically for the presence of calcium deposits. When calcium was found it was always within the caseous areas either as compact masses or frequently as scattered coarse granular deposits. Inasmuch as spontaneous calcification of tubercles is frequently seen in non-injected rabbits with tuberculosis of long duration, all studies were carried on in animals that had the disease for not longer than 4 to 6 weeks. The number of calcified caseous tubercles per microscopic section were counted. The results are shown in Table I. It is clear that more (10 times as an average) caseous areas with calcium deposits were found in animals repeatedly injected with $CaCl_2$ than in non-injected animals. Furthermore the degree of calcification was generally more marked in the injected

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[7] Maver, M. E., and Wells, H. G., *Am. Rev. Tuberc.*, 1923, **7**, 1.

[8] Spies, T. D., *Am. J. Path.*, 1930, **6**, 337.

[9] Cameron, G. R., *J. Path. and Bact.*, 1930, **33**, 929.

TABLE I.
Calcification of Tubercles in Lungs of Animals Receiving Daily Injections of Calcium Chloride.

Non-injected Animals			Injected Animals			
Rabbit No.	Interval between injection of tubercle bacilli and death of animal	Number of caseous areas with calcium deposits per section	Rabbit No.	Interval between injection of tubercle bacilli and death of animal	Total amount of 5% $CaCl_2$ injected	Number of caseous areas with calcium deposits per section
	days			days	cc.	
1	26	0	8	27	32	3
2	28	1	9	28	32	2
3*	31	0	10	34	55	4
4	33	0	11	34	60	2
5	34	1	12*	36	19	7
6	34	1	13	43	19	7
7	37	0				
Average		0.43				4.1

* These 2 animals were not killed but allowed to die of tuberculosis.

than in the few non-injected rabbits, each of which showed 1 spontaneous calcified tubercle.

Further experiments are being conducted to substantiate these preliminary results by quantitative analyses and also to determine the effect of the accumulation of calcium in tuberculous areas on the course of development of the disease.

5644

Further Studies by the Angiostomy Method of Hormonal Secretion of Pancreas and Suprarenal.

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These experiments are concerned with the study of the effect of the administration of various substances upon the function of the pancreas and the adrenal.

The method employed consisted of the use of an angiostomy cannula implanted on the superior pancreatico-duodenal vein and the suprarenal vein. The use of this cannula had already been recorded.

Procedure. Three control samples of blood were drawn from the angiostomized vessels and from the femoral artery. 0.2 cc. of each sample of blood were then injected intraperitoneally into three white mice. This was followed by the injection of the materials studied into the femoral vein of a dog. At varying intervals after the intravenous injection, blood was again drawn from the three vessels and injected intraperitoneally into a new series of white mice. These mice were subsequently killed two hours later by decapitation and their blood sugar determined by the Hagedorn-Jensen method. The secretion of epinephrin and insulin was interpreted by the effect upon the blood sugar changes in the mice. The substances injected intravenously were dextrose, levulose, galactose, tyrosine, alanine and morphine. Similar experiments were performed on dogs fed milk and glucose.

The influence of levulose and galactose on epinephrin and insulin secretion proved to be similar to that of dextrose, both sugars calling forth insulin secretion and depressing the secretion of epinephrin. A similar result was obtained by feeding milk and dextrose.

For the first few minutes after tyrosine injection there was no change in the blood sugars of the mice. Thirty to 45 minutes later, the blood sugars rose and one hour and 45 minutes later there was a definite depression. Alanine had no effect. The most effective stimulant of epinephrine secretion appeared to occur after the injection of the morphine.

5645

Failure to Immunize the Monkey Against Poliomyelitis by Prolonged Nasopharyngeal Spraying with Live Virus.*

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In an attempt to explain the protection which a large number of children and the majority of adults enjoy against poliomyelitis it has been generally held that this immunity is acquired by repeated contact with subinfectious amounts of the active virus. It has also been suggested that this process of latent immunization is accomplished by small doses of virus entering the body through the upper respiratory tract. We have undertaken to verify experimentally the soundness of this hypothesis.

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* Under a grant from the International Committee for the study of infantile paralysis, whose work is being financed by Jeremiah Milbank.

Four rhesus monkeys were subjected to extensive spraying of the nose and throat with a 10% emulsion of live poliomyelitis virus during a period of about 2½ months. The treatments were given twice a week, so that each monkey received 20 sprays in all. The amount of virus consumed for each spray was approximately 1 cc. per monkey. At the end of the immunization period one of the 4 treated monkeys had died from extensive visceral tuberculosis; the autopsy showed a perfectly normal cord and histological examination revealed no lesions of poliomyelitis. From each one of the 3 remaining animals a sample of blood was obtained after a rest period of about one month following the last spray. These monkeys at the same time were inoculated intracerebrally with 1 cc. of a 10% virus emulsion to test for immunity, one normal control accompanying this test. All 3 treated monkeys developed typical poliomyelitis together with the control. Inasmuch as it has been suggested that the intracerebral inoculation is too severe a test to determine slight degrees of immunity, we have examined the sera of these 3 monkeys in the customary manner for the presence of virucidal substances. Equal quantities of serum and of 1% virus emulsion, 0.5 cc. in each case, were mixed, incubated for 2 hours at 37°C, left in the icebox overnight and the mixture, 1 cc., was then injected intracerebrally into a monkey; one control animal received a similarly prepared mixture of virus and salt solution. Although a very small amount of virus was deliberately chosen for this test, all monkeys in this experiment came down with typical poliomyelitis.

The experiment leaves no doubt that prolonged spraying of the nose and throat of the monkey with fairly large doses of active virus fails to induce any demonstrable immunity, as indicated by the lack of individual protection against brain infection and by the absence of virucidal substances in the blood serum. However, in view of the many differences in host susceptibility and mode of infection between the human disease and the experimental infection in the monkey, it is questionable to what extent these results are directly applicable to the problem of human epidemiology.

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The Flocculation of Lecithin by the Extracts of Adrenal Glands.

JIRÍ STEFL. (Introduced by Frank A. Hartman.)

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In a previous publication[1] I have described the following reaction: The lipid of adrenal glands is flocculated in an aqueous emulsion in the presence of living tissue, oxydized adrenalin and H_2O_2. After sedimentation of the floccules it is possible to determine the presence of cholin and phosphoric acid in the supernatant liquid. It was observed that this phenomenon occurs very quickly, especially at body temperature, that oxydized adrenalin is not necessary, and that H_2O_2 only increases the speed of this reaction but does not cause it. Instead of living tissue a native preparation of blood albumen was used, which was found to be necessary and sufficient.

In this way the reaction became very simple: A lipid emulsion of adrenals is flocculated in the presence of native blood albumen. Another lecithin (lecithin *ex ovo* Merck) did not show this reaction. A very important condition is the integrity of the albumen; if this is denaturated the reaction is negative. The optimal temperature is body temperature; lower temperature inhibits the reaction; a higher one causes a denaturation of the proteins, so that the flocculation does not appear. The albumen does not enter into the reaction with the lipids; neither is it destroyed. After the reaction and after filtration it is possible to denature the albumen or to use it for a new reaction. It acts probably as a catalyzer (ferment?).

It was always possible to determine after flocculation and filtration, a certain amount of cholin and phosphoric acid in the clear solution, which obviously originates from the decomposition of lecithin. But this decomposition is perhaps only an accompanying phenomenon; the main manifestation is the flocculation.

Further I investigated why only the lipids of the adrenals flocculate upon coming in contact with proteins, and other lecithins do not. There are two possibilities: Either the lecithin from the adrenals is different or it is accompanied by a compound, which is the real condition for the reaction as described above. I therefore prepared pure lecithin from the adrenal glands according to Erlandson, i. e., an alcohol extract is prepared, evaporated, the residue dissolved in ether and the concentrated ether solution precipitated with acetone. The solution in ether and the precipitation with acetone are repeated several times and both fractions, i. e., the precipitate and the solution were used. The precipitate which represents a lecithin of high purity after the evaporation of acetone was emulsified in water. The emulsion did not show the above described reaction. The acetone-ether solution was evaporated in a vacuum and the residue dissolved in water. This added to the lecithin emulsion of the adrenals and to the albumen immediately caused a flocculation of the lecithin. This flocculation in the presence of the acetone-ether solution appeared also if we used a lecithin of different origin (e. a. lecithin *ex ovo* Merck). It is apparent that the flocculating reaction is not caused by the lecithin but it depends on a compound in the adrenal glands which accompanies the lipids in their solvents.

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[1] *Ctp. rend. des séances de la Soc. Biol.*, **103**, 647.

We prepared an extract which prevents the going over of lipids of lecithin nature into the solvents and which at the same time denatures the proteins. From dissected adrenals an extract with saturated cadmium chlorate solution in 96% alcohol was prepared, after 24 hours the extract was evaporated in a vacuum, from the residue after removing the fats and cholesterin through simple filtration an ether extract was prepared. This ether extract was evaporated in a vacuum and the residue dissolved in water. The solution prepared in this way showed the above mentioned qualities. The substance itself is of medium hard consistency, yellowish color, does not crystallize, smells like acetic acid, has a slight acid reaction and gives a cacodylic reaction. On account of technical difficulties it was not possible to test it on epinephrectomized cats. The cortin prepared by Hartman also gives the flocculating reaction. Hartman and Brownell[2] prepared a substance from the adrenals by ether extraction which they call cortin and which keeps epinephrectomized cats alive.

A short time before Swingle and Pfiffner[3] prepared an extract by a more complicated process, which contained the cortical hormone and was shown to keep epinephrectomized cats alive in good condition. Concluding from the publications of the authors, their preparation has some of the same qualities as above described substance, in that it is soluble in solvents of lipids, unstable in alkaline solutions and gives as it was found only for cortin the flocculating reaction. Swingle and Pfiffner's cortical hormone was not examined because its preparation is very difficult and I could not obtain a market preparation.

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[2] Hartman and Brownell, *Am. J. Physiol.*, 1930, **93**, 655; PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 938.

[3] Swingle and Pfiffner, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 510; *Science*, 1930, **1**, 321.

If we assume that the cortical hormone regulates the relation between the lipids of the tissue and the medium, it is possible to suppose that the substance which flocculates the lecithin in the presence of albumen is, if not the same, closely related to the cortical hormone.

5647

Bubble Recorder for Mariotte Bottles.

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The recording of bubbles passing through a Mariotte bottle by means of a tambour offers considerable technical difficulties, as for instance, in the lung perfusion, described by Sollmann and von Oettingen;[1] nor does the device, worked out by Atzler and Frank[2] give satisfactory records.

Very good results have been obtained with the following device which utilizes the oscillation of the fluid in the inlet tube of the Mariotte stopper to close a circuit between a pair of wire electrodes adjusted in the lower end of the tube. The electrodes consist of copper wires fused into narrow glass-tubes for insulation, with projecting platinum tips. One wire is adjusted to about one millimeter above the lower rim of the tube, the other wire dips about 0.5 cm. into the fluid. The 2 electrodes are held together by a rubber-ring through which a pin passes, which rests upon the upper rim of the Mariotte tube (Fig. A). By shifting this ring up and down, the electrodes can be adjusted to different levels; they are connected with a current of 110 volts and with a relay arrangement,

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[1] Sollmann, T., and von Oettingen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 692.

[2] Atzler, E., and Frank, L., *Pflüger's Arch. ges. Physiol.*, 1920, **181**, 141.

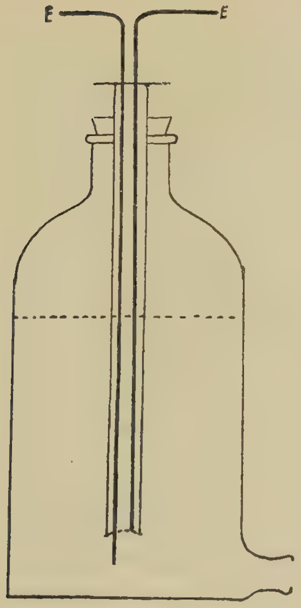

Fig. A

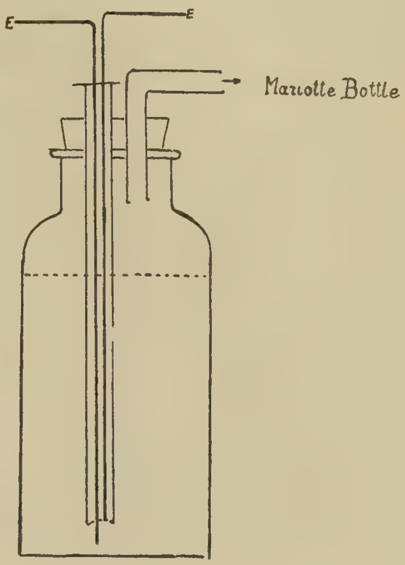

Fig. B

the dry cells of which feed a signal magnet, as described by Biskind and Dan.[3]

When the fluid within the Mariotte tube rises a little after each bubble, the 2 electrodes are short-circuited, and a signal mark is produced on a moving drum. There is virtually no danger of changing the composition of the saline solution by electrolytic decomposition products, because the contact between saline and electrodes is short, and the quantity of saline coming in contact is very small.

In case very unstable solutions and sensitive objects are used, the electrodes may be placed into a small supplementary bubbling bottle partly filled with saline solution, the air-chamber in the top of this bottle being connected with the inlet tube of the Mariotte bottle (Fig. B).

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[3] Biskind, M., and Dan, M., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 52.

5648

On the Nature of the Toxin-Antitoxin Neutralization Studied on Collodion Particles.

JULES FREUND.

From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

Diphtheria and tetanus toxins can be adsorbed upon collodion particles and can then be neutralized by the adsorption of their antitoxins.[1, 2] When, however, the particles were treated first with tetanus antitoxin and then with tetanus toxin a phenomenon, at first sight paradoxical, appeared, which may be interpreted as the combination of toxin with antitoxin without neutralization. Further observations are presented here.

Collodion particles prepared according to Loeb[3] and unconcentrated antitoxic horse serums* were used in 3 types of experiment: A—first treatment with the antitoxin, second treatment with the toxin; B—first treatment with normal serum or a non-corresponding antitoxin, second treatment with the toxin; C—treatment with the toxin alone.

Tetanus toxin. With antitoxin dilution 1:1 and toxin 1:1 or 1:10, and with antitoxin 1:10 and toxin 1:1 or 1:10, the mice in experiment B showed no symptoms at all; in experiments A and C they either died or had severe symptoms of tetanus. When antitoxin was used in dilution 1:10 and toxin 1:100, the mice in experiments A and B had no symptoms and in experiment C they showed symptoms of tetanus.

Diphtheria toxin. Collodion particles treated with diphtheria toxin alone produce a nodule in the skin of guinea pigs, and the skin over the nodule becomes red and scaley 2 or 3 days after the injection. Toxin + antitoxin produces a nodule over which the skin shows no signs of inflammation. With toxin + normal serum the reaction is the same as with toxin alone. If, however, the order of treatment is reversed, that is, if the particles are first treated with anitoxin and then with toxin, the skin in experiment A shows severe inflammation, and in experiment B no inflammation. In

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[1] Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 65.

[2] Freund, J., *J. Immun.*, 1931, in press.

[3] Loeb, J., *J. Gen. Phys.*, 1923, **5**, 109.

* The toxin and antitoxin preparations were obtained through the courtesy of Prof. K. F. Meyer of the George Hooper Foundation, and Dr. John H. Reichel of the H. K. Mulford Company.

repeated experiments the inflammation with particles treated with antitoxin + the corresponding toxin was strikingly more intense than with those treated only with toxin.

The results of the experiments are summarized as follows:

A tetanus antitoxin + tetanus toxin: toxic.
B diphtheria antitoxin + tetanus toxin: not toxic.
A′ diphtheria antitoxin + diphtheria toxin: toxic.
B′ tetanus antitoxin + diphtheria toxin: not toxic.

Two questions naturally arise. (1) Why are the particles that have been treated with a non-corresponding serum + the toxin, not toxic, when those treated with the corresponding antitoxin and toxin, are toxic? The latter observation, but not the former, could be satisfactorily explained as an adsorption of the toxins as well as of the antitoxins upon the collodion particles. In view of the results, however, the question can be answered by assuming that collodion particles treated with the non-specific serums adsorb or retain little if any of the toxin, whereas those treated with antitoxin are capable of combining with the corresponding toxin, as if in a reversed Castellani absorption experiment.

(2) Why are the particles, treated with antitoxin + toxin, toxic when the opposite order of treatment yields non-toxic particles? A tentative answer can be given. A combination between toxin and antitoxin is evidently not sufficient for neutralization. This conclusion is also supported by the fact that under certain conditions toxin can be recovered from toxin-antitoxin mixtures, suggesting that the neutralization following chemically specific combinations is a physical phenomenon. Neutralization takes place only when toxin and antitoxin are combined in a certain way. They are combined whatever the order of treatment of the collodion particles, but it has been seen that the toxin is neutralized only when the antitoxin is in the top layer, i. e. when the antitoxin covers the surface of the toxin molecule. Similarly Jones[4] has shown that collodion particles treated with precipitinogen + precipitin are flocculated, but not with the reverse treatment. Antitoxins adsorbed on charcoal are incapable of neutralizing their toxins.[5] These observations suggest that the toxin-antitoxin reaction is essentially similar to other antigen-antibody reactions, particularly precipitation, agglutination and phagocytosis.[6]

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[4] Jones, F. S., *J. Exp. Med.*, 1928, **48**, 183.

[5] Eisler, M., *Biochem. Z.*, 1923, **135**, 416; 1924, **150**, 350.

[6] Mudd, S., Lucké, B., McCutcheon, M., Strumia, M., *J. Exp. Med.*, 1930, **52**, 313.

This conclusion is strengthened by experiments showing that tannin adsorbed on red blood cells mediates agglutination, promotes phagocytosis, and prepares the cells for lysis by complement,[7] and that it combines with, and detoxifies, toxins adsorbed on collodion particles.

I am indebted to Miss Elizabeth S. Stivers for valuable assistance.

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Studies on Mouse Leukemia. IV. Specificity of Susceptibility to Different Lines of Inoculated Leukemia.*

E. C. MAC DOWELL AND MAURICE N. RICHTER.

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Susceptibility and resistance to a particular line of inoculable mouse leukemia may, for present purposes, be defined in terms of the presence or absence of conditions necessary for the survival of the active agent. A group of 11 such lines of agent, each of which originated in a different case of spontaneous lymphatic leukemia in a highly inbred strain of mice designated as C58, found the necessary conditions for survival in mice of the same strain.[1] Although different lines of agent can grow in the same pure bred strain of mice, these different lines do not all have the same requirements for survival, as has been shown by hybridization experiments.[2] The present report gives direct evidence of the specificity of requirements of different lines of agent, without the use of hybridization, by means of a new line that originated from a spontaneous case in another highly inbred strain of mice.

The new line of agent is designated line L. The spontaneous case that gave rise to this line was a mouse in strain 89; the line is carried in mice of this same strain. During the course of 13 trans-

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[7] Reiner, L., and Fischer, O., *Z. f. Immunitatsf.*, 1929, **61**, 317; Reiner, L., and Kopp, H., *Ibid.*, 1929, **61**, 397; Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1922, **26**, 876; Neufeld, F., and Etinger-Tulczynska, R., *Zentralbl. f. Bakt.*, 1929, **114**, 252.

* Aided by a grant from the Carnegie Corporation and an appropriation for technical assistance from the Research Fund of Columbia University.

[1] Richter and MacDowell, *J. Exp. Med.*, 1930, **51**, 659.

[2] MacDowell and Richter, *J. Cancer Res.*, 1930, **14**, 434.

fers 75 mice of this strain have been inoculated. Of these all but 4 (94.6%) have died with unmistakable lesions of leukemia, mostly between the 15th and 25th days after inoculation. The agent of line L has been inoculated into 64 mice of strain Storrs-Little with completely negative results and into 82 mice of strain C58, of which all but one were negative.

The active agent of line I originated in a spontaneous case in strain C58 in April, 1929. It has now reached its 116th transfer. Barring experiments designed to study the nature of the agent, 859 mice of this strain have been so inoculated; all have proved susceptible with the exception of a single mouse whose identity is questionable. A total of 225 mice of strain Storrs-Little have been inoculated with the agent of line I with no positive case. But a large proportion of the mice from strain 89 inoculated with agent of line I die with the lesions characteristic of line I (94 positive, 48 negative). The proportion of susceptible mice in this total has no significance since different matings within the strain give different proportions of susceptible animals. Only the study of individual families gives significance to the ratios obtained. This indicates that in spite of many generations of inbreeding by brother to sister matings, which usually renders a strain uniform genetically, strain 89 is at present not genetically homogeneous in regard to the susceptibility to line I, although to line L it is probably uniform.

To summarize, all families of strain 89 are susceptible to line L; some of them give numerous individuals susceptible to line I, others give few such individuals, while strain C58, on the other hand, is susceptible to line I and virtually negative to line L. The same animal may provide resistance, as well as different types of susceptibility.

It is not correct, therefore, to regard "susceptibility to inoculated leukemia" as a state dependent on any single set of conditions. The number of different sets of conditions that produce susceptibility depends on the number of lines of agent that may be found with differing requirements. Susceptibility can be defined only with reference to a particular line of agent.

5650

Afferent Impulses in the Carotid Sinus and Aortic Nerves.

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Afferent impulses in the carotid sinus nerve of the rabbit have been recorded by means of a vacuum tube amplifier and either a string galvanometer or Matthews oscillograph and the arterial pulse curve registered simultaneously with a Wiggers manometer. The experiments show that there is a burst of impulses accompanying each heart cycle followed by comparative inactivity. This discharge is coincident with the rapid rise in arterial pressure revealed by the carotid pulse curve. The duration of the nerve activity extends usually to about the incisura although frequently there are scattered impulses continuing throughout diastole. In some cases there is a second smaller outburst which coincides with the rise in pressure following the incisura. At high blood pressures the discharge becomes continuous, an effect which is likewise produced as a result of asphyxia.

The general character of the discharge in the carotid sinus nerve agrees closely with that found in the aortic (cardiac depressor) nerve. The impulses in this nerve have been recorded by Adrian[1] and several other workers and simultaneous records of the impulses and pulse curves have recently been reported by the author.[2] Here, too, there is a large outburst of impulses synchronous with the rapid rise in pressure the major part of the discharge usually continuing to the incisura. The activity of the sensory nerve endings in both the aorta and the carotid sinus appears, on the basis of these experiments, to be a function of both the absolute level of pressure and the rate of change of pressure.

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[1] Adrian, E. D., *J. Physiol.*, 1926, **61**, 49.

[2] Bronk, D. W., and Kaltreider, N. L., Montreal Meetings, Am. Physiol. Soc., 1931.

5651

Selective Susceptibility of the Epithelial Layer of Skin to Certain Bacterial Toxins.

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In connection with the histological study of skin reactions in hypersensitive animals, we studied microscopically the skin lesions produced by the injection of various substances both bacterial toxins and chemical irritants. We noticed an interesting characteristic of the lesions produced by staphylococcus and diphtheria toxins. The epithelial layer of the skin was destroyed early and extensively, out of proportion to the inflammatory reaction in corium, whereas, in the reactions of hypersensitive animals (excepting strong tuberculin types of reactions) and after injection of turpentine or pneumococcus emulsion, although the inflammatory reaction was strong, the epithelial layer was not apparently injured. The skin shows symptoms in several infectious diseases and in the hypersensitive state skin sensitiveness is usually marked. As the cause of this special susceptibility of the skin is not known, it seemed of interest to study a susceptibility which depends probably not on the reactions of mesenchymal but on the reaction of epithelial tissue. The early destruction of the epithelial layer has been known for a long time in lesions caused by the diphtheria bacillus and more recently Parker[1] observed necrosis of the epithelium in the case of staphylococcus toxin.

We examined the histological structure of skin reactions produced by staphylococcus and diphtheria toxins in 7 guinea pigs and 8 rabbits. In every animal several reactions were studied in different stages of development. The staphylococcus toxin, obtained according to the method of Burnet,[2] was quite potent as 0.001 cc. caused necrotic skin reactions in rabbits. After injecting 0.05 cc. of this toxin into the skin of a guinea pig, in a few hours a quite extensive slight swelling developed with moderate hyperaemia. The next day the swelling and hyperaemia decreased. The skin covering the bleb formed by the injection became after a few hours pale with a slight violet hue. Necrosis was obvious to the naked eye in the course of the following days, though in sections made only 3 hours after the injections, microscopic examination of the epithelium, corresponding to the discolored central area, already showed marked changes. The cytoplasm stained pink with the usual hemotoxylin and eosin stain and the nuclei were shrunken, pyknotic and often surrounded by vacuoles. We found at this stage a moderate edema and infiltration with white cells (mostly polymorphonuclear) in the deeper layers of the skin extending much farther than the area of necrotic epidermis. Six hours after the injection, there was no change in the sections. After 24 hours, the epithelial cells showed marked degeneration and in many places showed infiltration with polymorphonuclear leukocytes. A new layer of epithelium was growing in from both edges below the old necrotic layer. In the upper layers of the corium, adjoining the necrotic epithelium, there were several foci of dense infiltration with polymorphonuclear leukocytes. The epithelial cells of the hair follicles had either disappeared or their nuclei had become pyknotic and the cytoplasm acidophilic. Compared with the 6 hour period, the edema and infiltration of the skin outside of the area of the epithelial necrosis had decreased.

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[1] Parker, J., *J. Exp. Med.*, 1924, **40**, 761.

[2] Burnet, F. M., *J. Path. a. Bact.*, 1930, 331.

The reactions with diphtheria toxin developed more slowly and the necrosis of the skin was grossly visible only after 48 hours and increased in size in the following days. This destruction was visible microscopically in 24 hours and during the following days strong infiltration with leukocytes developed below the necrotic epithelium. Even with small doses of the toxin, the inflammatory reaction in the corium was stronger than in the staphylococcus lesions. In the sections it was evident that the area of necrosis of the epithelium extended far beyond the zone of marked reaction in the corium.

Our findings in rabbits were essentially the same as in the guinea pigs. The thinness of the epithelium, however, made it more difficult to observe the first alterations.

We call attention to the following characteristics of the histological observations, which are especially apparent in the staphylococcus reactions: The early development of the necrosis of the epithelium, the larger extent of the area of this necrosis than that of the strong reaction in the corium, and the development of marked infiltration with white cells consecutive to the necrosis of the epithelium. These characteristics of the histological picture strongly suggest that the properties of these skin reactions depend largely on the injury to the epithelium and the cause of the special susceptibility of the skin is probably the susceptibility of the epithelial layer.

5652

Nature of the Goiter Producing Substance in Cabbage.*

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In the course of our attempts[1, 2] to isolate the substance in cabbage that produces goiter in rabbits we found that: (1) Cabbage was entirely inactivated by drying in air at 25-35°C., or *in vacuo* at a higher temperature. (2) Cabbage extracted with various solvents such as ether, alcohol, acetone, etc., was quite inactive. (3) These extracts concentrated *in vacuo* and fed to rabbits were likewise ineffective.

Since the substance in whole cabbage is not injured by prolonged boiling in air, by acid and alkali hydrolysis, and by autoclaving at 150°C., we concluded that these negative results might be due to volatilization. During the past year we have tested this assumption as follows:

1. Four rabbits were fed on cabbage, steamed in bulk for 35 minutes; 4 additional rabbits were fed on raw hashed cabbage that was steamed in a thin layer for 35 minutes. The rabbits on cabbage steamed after hashing had hyperplastic thyroids after 4 weeks' feeding, but definitely less marked than those on cabbage steamed in bulk. 2. Attempts were made to distil cabbage in various ways and to feed the volatile portion. Some methods gave negative results. With others a product was obtained which caused slight thyroid hyperplasia. 3. Since the agent in cabbage that causes goiter could be removed by several organic solvents, and we thought it might be volatile, we next attempted to remove it with ethyl ether. After removal of nearly all of the ether at atmospheric pressure, the "cabbage fat" (ether extract) was fed to 4 rabbits for 30 days, in which time definite thyroid hyperplasia had developed, while an equal number of control animals showed normal thyroids.

Another lot of "cabbage fat" obtained from the ether extract was distilled. A small amount of the residue was fed daily for 5 weeks to 3 rabbits. Three other rabbits were fed with about 0.5 cc.

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* Aided by a grant from the Ella Sachs Plotz Foundation.

[1] Marine, D., Baumann, E. J., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 822.

[2] Marine, D., Baumann, E. J., Webster, B., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1025.

of the distillate daily for the same time. The distillate contained a considerable amount of ether and the amount eaten corresponded to the distillate from only one half the amount of residue that was fed. Three rabbits were used as controls. The diet of all was alfalfa and oats. The thyroids of the control rabbits were practically normal, while those of the animals receiving the "cabbage fat" residue were definitely hyperplastic as were also the thyroids of the rabbits fed on the distillate, although the amount of distillate fed was comparatively small.

These experiments we believe indicate that the goiter-producing substance in cabbage is extractable with ether and is volatile.

5653

Effect of Medulla Transplantation.

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The experiments recently reported on the transplantation of parts of the central nervous system indicate that cellular hyperplasia can be induced in regions of the cord which normally have a low motor content. Heterotopic transplantations of the cord show conclusively that extensive hyperplasia, both motor and sensory, can be obtained. Previous experimentation by the author[1] has indicated that, with the cord in its normal location, there is a direct dependent relationship between the cellular content of the trunk segments of the cord and that of the regions anterior to it. These experiments are in direct confirmation of Detwiler's results.[2]

The present series of experiments deals with the transplantation of an additional medulla just posterior to the normal medulla oblongata of the urodele embryo. A transverse section is made at the lower part of the medulla and the parts of the nervous system separated. The transplant which includes the ear capsules with the portion of the medulla in close association with them is then placed between the separated parts. The tissue contains Mauthner's cell area and the regions of the ninth and part of the tenth cranial nerves.

The transplanted ear capsules fuse with the normals, giving rise to a large mass of cartilaginous tissue which increases the proportions of the head and apparently permits the interpolated portions to develop. The ganglionic elements of the 9th and 10th fuse in a massive ganglion which is 40% larger than normal, but is smaller than the double ganglion content which would be expected. The nerves arising from the fused 9th and 10th form regular but enlarged trunks with regular distribution, containing, however, a greater number of fibers than are normally found in these nerves.

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[1] Nicholas, J. S., *Roux's Arch. f. Entw. Mechanik*, 1929. Bd. 118.

[2] Detwiler, S. R., *J. Exp. Zool.*, 1925, **41**.

A careful study has been made of the distribution of the cellular and fiber contents of the cord lying posterior to the region of the transplantation in 26 cases from a series of 200 animals operated upon. The relations of the cells and fibers as indicated by the percentage of the total area which they occupy is remarkably regular. The motor areas are increased in size and in the number of cells which are found within them. The sensory elements have been displaced dorsally in the cord and due to their compactness show an increase in the number of fibers found in the dorsal fiber area.

The conclusion is drawn from the above experiments that a quantitative increase in the number of cells anterior to the spinal regions of the cord due to the presence of a small part of the posterior region of the medulla will produce a definite hyperplasia in the regions behind the graft. Detwiler has shown that the sensory cells respond primarily to the changes in the peripheral field and that motor hyperplasia of the cord in the normal position is due to intrinsic influences. In the present report a small segment of the medulla involving none of the spinal segments can cause, when transplanted, a hyperplasia of the ventral area of the spinal cord. Since this effect is not nearly so marked as when a larger transplant is used, as in Detwiler's cases, the result must be interpreted as due to the quantitative increase of cells and fibers contained in the additional medulla oblongata.

5654

Imbibition in Disintegration.

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From the Osborn Zoological Laboratory, Yale University.

Studies of the lethal effects of cyanide solutions on *Amblystoma punctatum* embryos have demonstrated that water relations play an important rôle in their subsequent disintegration. The manner in which this water is taken up, whether by osmosis or imbibition, has received little attention. The separate evaluation of water in these

2 rôles appears essential to a basic understanding of the disintegration process.

Measurements of density should help to reveal the nature of the water entering the tissue for it is generally recognized that water of imbibition increases in density whereas water taken up osmotically does not increase in weight per unit volume. An embryo imbibing water will increase its weight when weighed in water and will at the same time decrease the total volume of embryo plus solution. An embryo taking up water by osmosis, however, may increase its own volume but the total volume of the embryo and solution will remain constant. Water taken up in this manner will not alter the weight of the embryo when weighed in water. To determine the amount and rate at which *Amblystoma punctatum* embryos took up water by imbibition, the following method was used:

A small Gooch crucible was suspended by a fine wire from one of the hooks of an analytical balance. A beaker containing the cyanide solution (M/125,000 in tap water) was placed beneath the crucible so that it hung completely immersed in the solution. Weights were hung from the hook above the crucible to balance the pan on the side opposite.

The embryos used were in the yolk plug stage enclosed only in the vitelline membrane. Twenty-five were introduced into the crucible in each trial. The weight of the embryos was then recorded as quickly as possible and at intervals of one-half hour thereafter until disintegration began. An increase in weight was observed after a few minutes. The increase was most rapid during the first hour, then slowed gradually. A few moments after the embryos ceased to gain in weight they began to disintegrate, at which time there was a decrease in weight. Embryos at the same stage were weighed in tap water as controls. These controls gave no evidence of disintegration and made no increase in weight in 4 hours. In all these trials the temperature was maintained constant at 20°C.

These observations suggest that the pressure due to imbibed water may be a positive factor in disintegration; and that cyanide solutions in some way increase the ability of embryos of *Amblystoma punctatum* to imbibe water.

5655

Effect of Adrenal Cortical Hormone upon Respiratory Metabolism of Normal, Thyroidectomized and Thyroidectomized-Adrenalectomized Cats.*

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From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and Princeton University.

We have reported[1] the effect of bilateral adrenalectomy upon the respiratory exchange of cats, together with the changes in metabolism produced by the administration of the adrenal cortical hormone to these animals. The present study is a continuation of that work. The adrenal cortical hormone[2] and the method of measuring the respiratory exchange[3] are the same as were used previously.

The cortical hormone was injected subcutaneously in quantities of from 6 to 15 cc. daily into normal cats for periods varying from 2 to 10 days. Measurements of the respiratory exchange were made 18 hours after the injections. In a series of 12 such experiments, no appreciable constant variation from normal occurred. Further, the general health of the animals did not appear to be affected. Similar experiments were carried out in 3 normal rabbits with essentially the same result.

The normal level of respiratory metabolism was determined in a series of 5 cats. Total thyroidectomies were then performed. The metabolism gradually fell to a level varying between 20 and 25% below normal. The animals gained in weight and showed general symptoms of thyroid insufficiency. At this point, the cortical hormone was injected subcutaneously in quantities varying from 10 to 50 cc. over a time period of from 1 to 3 days. Within 24 hours after the injection, there was an increase in respiratory metabolism of from 15 to 30%. This change occurred in 80% of the experiments and continued for from 24 to 48 hours after the administration of hormone was discontinued.

It was considered advisable to determine whether or not the thyroid gland was a factor in bringing about the elevation of metabolism which occurred when the adrenal cortical hormone was in-

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* Aided by grants from the Josiah Macy, Jr., Foundation and Mrs. R. E. Breed.

[1] Swingle, W. W., Pfiffner, J. J., and Webster, Bruce, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 728.

[2] Pfiffner, J. J., and Swingle, W. W., *Am. J. Physiol.*, 1931, **96**, 180.

[3] Marine, D., *J. Metab. Res.*, 1922, **2**, 29.

jected into bilaterally adrenalectomized cats. Accordingly, the previously thyroidectomized cats used in the above experiments were bilaterally adrenalectomized in 2 stages. The respiratory exchange was measured daily. As was the case in the animals in which the thyroid was intact,[1] the metabolism fell with the development of the symptoms of adrenal insufficiency. When these symptoms became severe, injections of cortical hormone were begun. The metabolism rose promptly to a level slightly above the normal and remained there as long as the cortical hormone was administered. In other words, the changes in the respiratory exchange following bilateral adrenalectomy in cats are essentially the same, whether the thyroid is intact or totally removed.

5656

Effect of Injections of Cortin on Resistance of Suprarenalectomized Rats to Large Amounts of Histamine.

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In previous experiments it was demonstrated that repeated injections of cortin raise the resistance of suprarenalectomized rats to typhoid vaccine.[1] Similar results were obtained by Hartman and Scott.[2] In subsequent experiments we found that repeated daily injections of cortin raise the resistance of suprarenalectomized rats to 4 and 5 lethal doses of histamine (about 500 mg. of histamine per kg. of body weight).[3] In the following experiments an effort was made to increase further the resistance of suprarenalectomized rats to very large amounts of histamine, by injections of cortin. It has been demonstrated that adult suprarenalectomized rats are killed by 100 to 120 mg. of histamine per kg. when the drug is administered on the sixth day after suprarenalectomy;[4] that immature suprarenalectomized rats are killed by 150 to 200 mg. of histamine[3]; that normal rats are killed by 700 to 900 mg. of histamine per kg. of body weight.[4, 5, 6] In spite of the fact that rats have accessory cortical tissue, it has been the experience in this laboratory that all rats are highly susceptible to the effects of small amounts of histamine injected after removal of the suprarenals, and that if the histamine is administered from the 6th to the 14th day, 100% of rats will succumb to less than 200 mg. of histamine per kg. of body weight. In these experiments the cortical extract used was made in the laboratory according to the method of F. A. Hartman. Cortin is a potent extract of the suprarenal cortex. It is free from toxicity and epinephrin.

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[1] Perla, David, and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 648.

[2] Scott, W. J. M., and Hartman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 649.

[3] Perla, David, and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 650.

[4] Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

It was found that a batch of cortin can be assayed by estimating the amount of cortical extract injected intraperitoneally into suprarenalectomized rats on the 5th and 6th day after operation necessary to protect these rats against 200 mg. of ergamine acid phosphate.

Suprarenalectomized adult rats were injected daily from the day of operation with 1.5 to 2 cc. daily of cortin (1 cc. was the equivalent extractive of 40 gm. of cortex). On the 6th day after operation 5 rats were injected with 600 mg., 3 with 700 mg. and 8 with 800 mg. of histamine per kg. of body weight. Three normal rats received 900 and 950 mg. of histamine per kg. of body weight. Of the 5 rats injected with 600 mg. of histamine per kg. all survived. Of the 3 rats injected with 700, 2 survived and of the 8 receiving 800, 3 survived. The normal rats were killed with 950 mg. per kg.

TABLE I.
Effect of repeated injections of cortin on resistance of adult suprarenalectomized rats to histamine administered on the 6th day after operation.

No. of rats	Cortin Days after suprarenalectomy						Histamine mg. per kg. 6th day	No. Survived	No. Died
	1	2	3	4	5	6			
5	2 cc.	2 cc.	2 cc.	2 cc.	2 cc.	2 cc.	600	5	0
3	”	”	”	”	”	”	700	2	1
8	”	”	”	”	”	”	800	3	5
3 normals	0	0	0	0	0	0	900	2	0
3 ”	0	0	0	0	0	0	950	1	2

From these results it is apparent that it is possible to raise the resistance of suprarenalectomized rats in many instances almost to the normal level of resistance of normal rats by repeated injections of large amounts of cortin. The effect of cortical hormone on the resistance of suprarenalectomized rats is a specific

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[5] Crivellari, C. A., *Compt. rend. Soc. biol.*, 1927, **96**, 223.

[6] Voegtlin, C., and Dyer, H. A., *J. Pharmacol. and Exp. Therap.*, 1925, **24**, 101.

one. Previous work has shown that large amounts of saline have no demonstrable effect on the resistance of suprarenalectomized rats. Repeated injections of epinephrin have only a very slight effect.[8] An extract of the spleen made in the exact manner as the cortical extract failed to produce any increase in resistance of suprarenalectomized rats. The female sex hormone of Doisy (Theelin, Parke, Davis and Co.) did not raise the resistance of suprarenalectomized rats. Through the kindness of Prof. Harrow of the College of the City of New York, we tested the effect of the male sex hormone on the resistance of suprarenalectomized rats with negative results. We believe, therefore, that the action of cortin on the resistance of suprarenalectomized rats to histamine and other toxins and poisons is a measure of specific activity of the life prolonging hormone of suprarenal cortex.

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A Substance in Urine of Normal Human Adults That Raises the Resistance of Suprarenalectomized Rats.

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Though potent extracts of the suprarenal cortex as made by F. A. Hartman[1] and Swingle and Pfiffner[2] will prolong the life of suprarenalectomized cats, enormous quantities of suprarenal glands are necessary for the recovery of small amounts of the active substance. A more efficient method should be found for obtaining the substance in larger amounts in order to make the use of cortical hormone available.

It occurred to us that the cortical hormone may be eliminated in the urine in large amounts. A lipoid extract of the urine of normal young adults, males and females, was made. The urine of young male and female adults is extracted with benzene. The benzene is allowed to separate. The benzene fractions are evaporated at 40° *in vacuo* to dryness, the residue taken up in ether, the ether evaporated off *in vacuo* and the residue taken up in water or oil. The final

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[7] Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.

[8] Perla, David, and Marmorston-Gottesman, J., *Am. J. Phys.*, 1929, **89**, 152.

[1] Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.

[2] Swingle, W. W., and Pfiffner, J. J., *Am. J. Phys.*, 1931, **96**, 153.

aqueous product is colorless and turns faintly pink in 24 hours. There is no epinephrine present. It is non-irritating when injected subcutaneously or intraperitoneally and has no odor of urine. The procedure takes 36 to 48 hours. The final product was made up so that 1 cc. of the extractive was equivalent to 300 cc. of urine.

The effect of repeated daily injections of the extract on the resistance of suprarenalectomized rats to histamine poisoning was determined in the same manner and under the same conditions as extracts of the suprarenal gland in our previous experiments[3, 4, 5] with cortin prepared from the cortex of the whole gland according to the method of F. A. Hartman. Repeated injections of cortical hormone raise the resistance of suprarenalectomized rats to histamine poisoning administered on the 6th day after the operation. An arbitrary standard unit was established as that quantity of cortex administered on the 5th and 6th day after suprarenalectomy necessary to protect these rats against 200 mg. of histamine per kg. The minimal lethal dose of histamine for untreated suprarenalectomized adult rats is 100 to 120 mg. per kg. when given on the 6th day after suprarenalectomy.[6]

Thirty suprarenalectomized, 3 months old rats were used. Three received daily injections of 1 cc. of the urine extract from the first to the 6th day after operation. On the 6th day, these received 200 mg. of histamine per kg. Two survived and one died. Three rats received daily injections of 1 cc. of the urine extract and on the 6th day were given 300 mg. of histamine per kg. Of these, 2 survived and one died. Six rats received 1 cc. only on the 5th and 6th day after the operation and were given 200 mg. of histamine per kg. Four survived and 2 died. Five rats received 2 cc. only on the 5th and 6th day after operation and were given 200 mg. of histamine per kg. All survived. Two were given 2 cc. of extract on the 5th and 6th day and were injected on the 6th day with 300 mg. of histamine. One died and one survived. Two suprarenalectomized rats received 2 cc. of extract on the 5th and 6th day and were given 400 mg. of histamine. One died and one survived.

As controls, the protective effect of the male sex hormone of Funk and Harrow[7] was tested. We obtained a small quantity of the

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[3] Perla, David, and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 648.

[4] Perla, David, and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 650.

[5] Marmorston-Gottesman, J., and Perla, David, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1022.

[6] Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

[7] Funk, C., and Harrow, B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 325.

male hormone through the courtesy of Prof. Harrow. Four rats injected daily with 1 cc. of the extract were given 200 mg. of histamine on the 6th day. One rat survived 48 hours and 3 died within 8 hours. Two rats were injected daily with 10 units of "theelin," the female sex hormone of Doisy, and both died with 200 mg. of histamine per kg. Five suprarenalectomized rats were untreated and were given 200 mg. of histamine on the 6th day after operation. They all died.

TABLE I.
Effect of repeated injections of an extract obtained from urine on resistance of suprarenalectomized rats to histamine poisoning.

No. of rats	Sex		Extract of Urine* (days)						Histamine (mg. per kg.)	No. Survived	No. Died
	M	F	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.			
3	2	1	1	1	1	1	1	1	200	2	1
3	1	2	1	1	1	1	1	1	300	2	1
6	3	3	0	0	0	0	1	1	200	4	2
5	3	2	0	0	0	0	2	2	200	5	0
2	1	1	0	0	0	0	2	2	300	1	1
2	1	1	0	0	0	0	2	2	400	1	1
Funk-Harrow Male Hormone†											
4	2	2	1	1	0.5	0.5	1	1	200	1§	3
Theelin (Doisy Female Sex Hormone)‡											
2	1	1	10un.	10u.	10u.	10u.	10u.	10u.	200	0	2
Untreated Controls											
5	2	3	0	0	0	0	0	0	200	0	5

* 1 cc. equals extractive of 300 cc. of urine of young normal adults.
† 1 cc. equals extractive of 400 cc. of urine of adult males.
‡ Expressed in Allen Doisy rat units.
§ This animal survived 48 hours.

Comparing the results with extracts of suprarenal cortex with these experiments, it is estimated that one liter of urine furnished an equivalent amount of protective substance in about ½ pound of whole gland.

The extractive obtained from the urine contains a substance that has some of the physiological properties of cortex of the suprarenal gland. In previous experiments we found that an increase in resistance in suprarenalectomized rats following administration of cortin is a specific one. As with the cortical preparations, young adult suprarenalectomized rats injected with the extract from the urine gained from 8 to 10% in weight during the first week following suprarenalectomy. The suprarenalectomized untreated rats either lost weight or their weight remained stationary.

Preparations made from other organs such as the spleen in the manner of cortin; and theelin, the female sex hormone of Doisy, and the male hormone of Funk and Harrow, fail to raise the resistance of suprarenalectomized rats.

Experiments are in progress to determine the effect of this extract of the urine on suprarenalectomized cats.

A substance has been extracted from the urine of young human adults which has some of the physiological properties of the cortical hormone of the suprarenal gland.

5658

Experimental Inoculation of Man and Guinea Pigs with the Virus of Cattle Warts.

MALCOLM H. SOULE AND EARL B. MC KINLEY.

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The etiological agent of human verrucae has been established as a filterable virus by a number of investigators. This was suggested first by Ciuffo[1] and firmly established by Wile and Kingery[2] and by Kingery.[3] Schultz[4] reported that he had succeeded in transmitting cattle warts to man but the filterable nature of the cattle wart virus was not proven until the work of Creech.[5] Creech succeeded in producing experimental wart lesions in 15 out of 22 calves with both filtered and unfiltered cattle wart material.

It is becoming more and more apparent that there are many similar diseases to be found in the different species. Studies should be directed towards the discovery of common etiological agents in many of these conditions. The so-called pox diseases of man and animals represent good examples which suggest common or at least closely related etiological factors. Information concerning the relationship of these agents is particularly valuable.

We have attempted to determine a possible relationship between the virus of cattle warts and of the common wart in man. Usually

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[1] Ciuffo, G., *Gior. Ital. delle Malattie Venere e della Pelle,* 1907, **48**, 17.

[2] Wile, U., and Kingery, L. B., *J. Am. Med. Assn.,* 1919, **73**, 970.

[3] Kingery, L. B., *J. Am. Med. Assn.,* 1921, **76**, 440.

[4] Schultz, F., *Deut. Med. Wchnschr.,* 1908, **34**, 423.

[5] Creech, G. T., *J. Agric. Res.,* 1929, **39**, 723.

4 or 5 cattle warts have been excised, brought to the laboratory, washed and placed in 50% glycerin in the ice box for several hours. These warts were then cut up in fine particles with sterile instruments and ground up in sterile test glasses with sand. After emulsifying with saline part of the emulsion was filtered through Berkefeld N filters. Human subjects and guinea pigs were inoculated intracutaneously with the wart emulsions.

One of us (McK) had demonstrated that unfiltered cattle wart emulsion gave rise in guinea pigs to new growths of the proliferating type characteristic of verruca. The experimental lesions were not so marked with the filtered material although mild lesions were produced. In 2 human subjects no lesions were produced even after one year incubation.

Recently we have studied this question again, utilizing the same methods as before except that a larger number of cattle warts have been used in the preparation of the virus emulsions. Twelve or fifteen of these lesions have been obtained and the freshest of them have been employed for the inoculation experiments. The emulsions were highly concentrated. Three human subjects, including ourselves, were inoculated intradermally and over 20 guinea pigs were injected with the same material. Suggestive lesions were again obtained in guinea pigs but none of the 3 human subjects have shown any signs of lesions after nearly 5 months' incubation. Creech[5] reports that the experimental lesions obtained by him in young calves appeared usually within the second or third month. Wile and Kingery[2] report an incubation period of from 4 to 7 weeks with experimental warts in man although second generation lesions required nearly 6 months according to Kingery.[3]

Our experiments so far would indicate no relation existing between the viruses of cattle warts and human warts in spite of the positive results obtained by Schultz.[4] However, since these experiments are based presumably upon a single strain of cattle warts (coming from one herd) this should not be taken as final evidence that the viruses of both human and cattle verruca are not biologically related.

5659

On the Mechanism of Action of Anayodin (Yatren; 5 Iodo, 8 Oxyquinoline, 7 Sulfonic Acid).

F. W. VON OETTINGEN AND E. E. ECKER.

From the Department of Pharmacology and the Institute of Pathology, Western Reserve University.

Makarowa and Zeiss[1] showed that in animals sensitized to serum proteins anaphylactic shock could be prevented if a serum containing 2% Germanin were used for the shock injection. Steppuhn, Zeiss and Brychonenko[2] also have reported the formation of such denatured proteins in the presence of Germanin. It appears, from the studies of Roehl,[3] that the sulfonic acid groups are involved in this reaction. Since the clinical picture following the intravenous injection of Anayodin (Yatren) resembles a non-specific protein reaction, it seems possible that a similar reaction occurs *in vivo*. The mechanism in this case would be a chemical alteration of a fraction of body proteins resulting in the production of a foreign protein.

The experiments herewith reported were conducted to ascertain the influence of this chemical substance in anaphylaxis both in sensitizing and shock injections.

One series of 6 guinea pigs were sensitized by subcutaneous injections of one cc. of 0.5% solution of crystalline egg albumin $F_2P_2B_1$.* At the same time the second series of 7 animals received the same dose of a solution of crystalline egg albumin containing 1% Anayodin. Each series was then divided into 2 groups. Of the first series sensitized with egg albumin 3 animals were shocked with egg albumin and 3 with egg albumin and Anayodin. Of the second series sensitized to egg albumin and Anayodin 3 were shocked with egg albumin and 4 with egg albumin and Anayodin. Thus cross experiments with the various combinations were obtained. The results are presented in Table I.

Summary: Allowing for individual animal variability as a factor in anaphylactic shock, as a whole it may be said that in our

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[1] Makarowa and Zeiss, *Ztsch. f. Immunitatsf.*, 1923, **47**, 110.

[2] Steppuhn, Zeiss and Brychonenko, *Arch. f. Schiffs u. Tropen Hyg.*, 1923, **27**, 206.

[3] Roehl, *Arch. f. Schiffs u. Tropen Hyg.*, 1926, **30**, (Beiheft) 103.

* For the purified egg albumin we are indebted to Dr. A. G. Cole.

TABLE I.

I. SENSITIZED TO EGG ALBUMIN.

(a) Shocked with Egg Albumin.

No.	Weight gm.	Dose cc.	Effect
1	340	1	Gasping, convulsions, in 2 min. Collapse in 4 min. Dead—butterfly lungs.
2	295	0.5	After 2 min. dyspnea, 4 min. side position, 7 min. slight convulsions—recovered.
3	230	1	Immediate gaspings, convulsions, in 1 min. dead —butterfly lungs.
(b) Shocked with Egg Albumin and Anayodin.			
4	260	1	After 4 min. dyspnea, 5 min. scratching—survived.
5	250	1	Rapid respiration, 28 min. side position, after 42 min. dead—very slight emphysema.
6	240	1	1 min. convulsions, after 5 min. dead—butterfly lungs.
II. SENSITIZED TO EGG ALBUMIN AND ANAYODIN. (a) Shocked with Egg Albumin.			
7	250	1	Immediate dyspnea, convulsions, side position. dead after 5 min.—butterfly lungs.
8	220	1	Cough, dyspnea—recovery.
9	315	1	Gasping, convulsions, after 4 min. dead—butterfly lungs.
10	215	1	Marked dyspnea, gasping, nervous, recovery.
(b) Shocked with Egg Albumin and Anayodin.			
11	335	1	Dyspnea, convulsions, in 3 min. dead—butterfly lungs.
12	245	1	Rapid respiration, 4 min. highly dyspneic, 5 min. nervous—survived.
13	234	1	Gasping, convulsions, in 2 min. dead—butterfly lungs.

group of 13 animals Anayodin (Yatren) has no clearly demonstrable influence in preventing either sensitization or shock.

5660

An Improved Direct Method for Obtaining the Total White Cell Count in Avian Blood.

BRUCE K. WISEMAN. (Introduced by Charles A. Doan.)

From the Department of Medical and Surgical Research, Ohio State University.

Because of the normal presence of nucleated erythrocytes and thrombocytes in avian blood, it has always been difficult to count accurately all of the white blood cells. For this reason, most direct methods have been based upon some technique providing for the recognition of specific types of cells in the counting chamber and then, from the percentage of these types found in the differential count made upon the stained smear, the total number of white cells per cu. mm. of blood have been calculated. To distinguish between the various types of white blood-cells, previous investigators have used a solution of vital neutral red alone,[1] vital neutral red with formalin in a separate solution,[2] or neutral red with a separate solution containing formalin plus crystal violet.[3] Neutral red, however, stains both granulocytes and monocytes, and it is often difficult to distinguish the one from the other under the powers of magnification that can be used in conjunction with the counting chamber. On the other hand, if one attempts to include both of these cell types in the count, the wide variation in staining intensity of the monocytes frequently occasions inaccuracies through the missing of individual cells. This is especially a source of error when the counting of blood cells is assigned to technicians. Moreover, it was found that the diluting fluids which do not contain a fixative frequently fail to preserve the red blood cells intact, the resulting hemolysis making a further complication in arriving at accurate counts of both the red and white blood cells.

The fact that the predominating granulocyte in avian blood contains eosin-staining granules suggested the use of the dye given below, which, when combined with a fixative to preserve the erythrocytes, stains these granular cells so as to make them stand out sharply and distinctly in the counting chamber. The solution now being used in this laboratory has the following formula: Phloxine 50 mgm., Formalin 5 cc., Ringer's Solution 95 cc.

This solution does not deteriorate and preserves the red blood cells indefinitely. Used as a diluting fluid, it stains the eosinophilic granules a brilliant red, imparting to these cells a distinguishing characteristic that is in marked contrast to the remainder of the cells seen in the counting chamber. All other cells take the stain much less brilliantly and are not to be confused with the eosinophilic leucocytes. The concentration of phloxine as given is such as to cause maximum staining within one hour. More dilute solutions (3 mg. dye in 100 cc. solution) stain these cells almost specifically, but the time interval required is at least 3 hours. Where time is not a factor of importance, the weaker solutions are recommended as the contrast between eosinophilic leucocytes and all other cells in the counting chamber is then optimal.

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[1] Forkner, C. E., *J. Exp. Med.*, 1929, **50**, 121.

[2] Blain, D., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 594.

[3] Shaw, A. F. B., *J. Path. and Bact.*, 1930, **33**, 833.

The blood, obtained from the wing-vein of the bird, is diluted 1-200 in the red blood cell pipette with this fluid. After standing the required interval, the pipette is then shaken for 2 to 3 minutes, the counting chamber filled in the usual manner, and the count made, using an 8 mm. objective, a 10X ocular, and a strong white light for illumination. The entire ruled area is examined, counting only the brilliantly red-stained eosinophilic leucocytes. This figure is used for calculation of the total white blood cells after the percentage of eosinophiles is obtained from the differential count taken

TABLE I.

Numerical Factors for Use in Obtaining Total White Blood cells per cu. mm. Avian Blood.

Eosinophiles %	0	1	2	3	4	5	6	7	8	9
0	—	22222.2	11111.1	7407.3	5555.5	4444.4	3703.6	3174.5	2777.7	2469.1
10	2222.2	2020.1	1851.8	1709.3	1587.2	1481.4	1388.8	1307.1	1234.5	1169.5
20	1111.1	1058.1	1010.0	966.1	925.9	888.8	854.6	823.0	793.6	766.2
30	740.7	716.8	694.4	673.3	653.5	634.9	617.2	600.5	584.7	569.7
40	555.5	542.0	529.0	516.7	505.0	493.8	483.0	472.8	462.9	453.5
50	444.4	435.7	427.3	419.2	411.5	404.0	396.8	389.8	383.1	376.6
60	370.3	364.2	358.4	352.7	347.2	341.8	336.6	331.6	326.7	322.0
70	317.4	312.9	308.6	304.4	300.2	296.2	292.3	288.5	284.8	281.2
80	277.9	274.3	271.0	267.9	264.5	261.4	258.3	255.4	252.5	249.6
90	246.9	244.1	241.5	238.9	236.4	233.9	231.4	229.0	266.7	224.4
100	222.2	—	—	—	—	—	—	—	—	—

Explanation: The values given in the body of the table are the corresponding factors for the percentage of eosinophiles given at top and side. To obtain the total white blood cells per cu. mm. of avian blood, multiply the number of eosinophiles obtained in the diluting fluid (1-200) in counting the entire ruled area of the counting chamber (0.9 cu. mm.) by the factor in this table corresponding to the percentage of eosinophiles as obtained by examination of the stained smear. Suitable accuracy (less than 100 cells) can be obtained by omitting the decimal from these factors.

at the same time. The red blood cells may be counted from the same preparation in the usual manner.

Derivation of the figure representing the total white blood cells per cu. mm. of blood depends upon calculating the number of eosinophiles in one cu. mm. diluting fluid (× 10/9) then correcting for the dilution (× 200) and finally multiplying this result by the fraction $\frac{100}{\text{percentage of eosinophiles}}$.

Since for any particular total cell count these values are all constants with the exception of the percentage of eosinophiles, it is possible to calculate a single factor for use with each percent of eosinophiles. This simplifies the above calculations to one step, namely, the multiplication of the number of eosinophiles counted in 0.9 cu. mm. diluted blood by the factor corresponding to the percentage of eosinophiles found in the differential count. Table I contains 100 factors each corresponding to a particular percentage of eosinophiles.

Two examples will clarify this explanation:

Example 1. *Without Use of Table:*

Number of cells counted in entire ruled area (0.9 cu. mm.) 26
Percentage of eosinophiles in differential count 60
Total white cells $26 \times 10/9 \times 200 \times 100/60 = 9{,}627$

Example 2. *With Use of Table:*

Number of cells counted in entire ruled area (0.9 cu. mm.) 26
Percentage of eosinophiles in differential count 60
Total white blood cells $26 \times 370.3 = 9{,}627$

It is believed that both technical and mathematical errors will be reduced in observations on avian blood by the adoption of the procedures outlined in this presentation.

5661

Preliminary Observations on Human Blood in Early Syphilis by the Supravital Method.

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Cells in the circulating blood frequently reflect the pathological process which is occurring in the tissues. Reports of cellular changes in tissues of syphilitic rabbits have appeared recently.[1, 2] Preliminary blood studies in selected patients at the University Clinic showing early syphilitic lesions revealed such definite deviations from the normal differential blood count that it seemed worth while to make repeated observations on such cases. Seventeen young adults of both sexes with uncomplicated early syphilis were selected. Observations were made at weekly intervals for 3 weeks to 4 months. Standardized pipettes were used and the supravital technique utilizing Janus green and neutral red in physiological concentrations was used for differentiating the white blood cells.

The physiological range for the white blood cells in the normal adult human during any 24 hours represents a fluctuation of 100% in the total cells, with corresponding numerical changes in the individual cell strains. This normal qualitative and quantitative range must be recognized and only those deviations outside this range may be interpreted as related to specific tissue reactions.

In all but 3 cases, the total white cell count remained within normal limits, i. e., 5,000 to 10,000, throughout the period of observation. That the total number of white cells remains so consistently within normal limits may account for the lack of significance which has been attributed in the past to cytological studies of the peripheral blood in syphilis. It is only when careful differential studies of the cells of the blood are made that certain significant changes are noted.

At the time these observations were being made, Pearce and Rosahn[2] published findings from a supravital study of early primary and secondary syphilitic lesions in rabbits. A transient increase in monocytes in the peripheral blood was found associated with a monocyte proliferation at the site of the lesions.

In our human cases 75% of the patients showing early lesions presented a monocyte rise greater than the physiological range.* For example, a white male, aged 21, with a chancre of 7 weeks' duration and a generalized maculo-papulo-pustular rash covering the entire body of 5 days' duration, showed monocytes approaching their peak. Within the following week, 18% of the 10,000 white blood cells in the peripheral circulation were monocytes (1800 per cu. mm.). Under antiluetic treatment there was a rapid fall in the monocytes during the following 12 days, to 200 cells. The total

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[1] Morgan, Hugh J., *Trans. Assn. of Am. Phys.*, 1930, **45**, 67.

[2] Pearce, L., and Rosahn, P. D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 654.

* Shilling gives 6% monocytes as the normal average for the human adult, which in absolute numbers would represent from 300 to 600 cells.

polymorphonuclear neutrophils and lymphocytes remained within the limits of normal during this time, though both showed a definite "shift to the left," in younger types within their respective cell strains. A definite monocyte rise of greater or lesser magnitude has been found in the majority of the cases thus far studied in the primary and secondary stages of the disease. There have been no qualitative changes noted similar to those described in tuberculosis.

Seventy-five per cent of the cases have shown a definite increase in eosinophilic leucocytes, one patient having had as many as 2500 eosinophils in a total count of 9,000 white cells. An occasional patient has shown a marked eosinophilia without a monocyte increase in the blood, though the rule has been to find both of these cell types increased. The average early case has shown from 2 to 12% eosinophils, the total number seldom going above 1,000 per cu. mm. Basophils were present in 11 cases, but in increased relative and absolute numbers in only 2 patients.

The neutrophilic leucocytes were affected less than any of the blood cells in both number and quality, though an occasional patient showed a "shift to the left" in the nuclear formula.

Only a rare clasmatocyte has been seen in the blood of the cases thus far studied.

The lymphocytic fluctuations varied from patient to patient. The majority of the cases showed the total lymphocytes to be well within the established limits of normal. Qualitative studies revealed some tendency to an increase in the younger cell types. Four of the cases showed a very definite lymphocytosis associated with young cell types.

All cases are being followed to determine if possible the significance of the variation in cellular response during the initial stages of the disease, and the effect of various types of treatment on the cellular picture and clinical course of the disease in individual patients.

5662

Effect of Hexylresorcinol upon Ascaris and Hookworm Eggs.*

H. W. BROWN. (Introduced by Paul D. Lamson.)

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Ascaris eggs from canine, porcine and human sources are noted for their resistance to many chemicals. Cram[1] in a summary of various workers' experiments shows that acids, alkalies and alcohols as well as many bactericidal agents do not prevent normal development of ascaris eggs. Of the active substances reported by Cram are carbolic acid which in 5% solution kills the pig ascarid egg in 10 hours, and cresol which in 3% solution kills them in 5 hours. The most active substance is probably "hyperactive" iodine which has been shown by Chandler[2] to kill ascarid eggs within 10 minutes.

Hexylresorcinol, a substance related to phenol, has been shown by Lamson, Brown, Ward, Robbins[3, 4] to be a very effective anthelmintic against both the dog and human ascarid. The purpose of this paper is to bring attention to the great activity of hexylresorcinol upon ascaris eggs (*Ascaris suum*).

Method of testing drug action upon eggs. Eggs were obtained by dissecting out the anterior 2 inches of adult ascaris uteri which were cut into short sections, placed in water and well shaken, to prevent large adherent egg masses. The hookworm eggs were obtained by macerating dog stools in saturated saline and looping off the upper layer containing the eggs which were washed in tap water. In both cases the eggs were placed in the solution to be tested and at intervals a sample was removed and the eggs washed several times, placed in flasks of tap water at room temperature, and examined from time to time to note their death or development to the embryonated stage. Controls in tap water were run with each experiment. The differentiation of live from dead eggs is sometimes difficult; the best criterion is their development when placed in a favorable medium. Occasionally large egg masses persisted despite vigorous

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* The funds for carrying out this work were given by the International Health Division of the Rockefeller Foundation.

[1] Cram, E. B., *J. Agr. Res.*, 1924, **27**, 167.

[2] Chandler, W. C., *Quart. Bull. Mich. Agr. Coll.*, 1924, Feb. 1.

[3] Lamson, P. D., Brown, H. W., and Ward, C. B., *North Am. Veterinarian*, 1931, January.

[4] Lamson, P. D., Brown, H. W., Robbins, B. H., and Ward, C. B., *Am. J. Hyg.*, 1931, **13**, 803.

TABLE I.
The Effect of Resorcinol and Hexylresorcinol Solutions upon the Eggs of *Ascaris suum.*
Eggs with outer albuminous coat intact.

Time in solution	1% Hexylresorcinol suspension	0.1% Hexylresorcinol	0.05%* Hexylresorcinol	1% Hexylresorcinol 1% KOH	S. T.-37	1% resorcinol
15 min.	All dead within 5 minutes	Embryonated Active	Embryonated Active	Embryonated Active	Embryonated Active	Embryonated Active
30 ”		Few dead	”	”	”	”
1 hr.		Maj. dead	”	”	”	”
2 ”		” ”	”	”	”	”
5 ”		All dead	”	”	”	”
15 ”		” ”	”	”	”	”
27 ”		” ”	Few dead	”	”	”
48 ”		” ”	” ”	”	”	”
100 ”		” ”	Many dead	”	”	”
196 ”		” ”	Maj. dead	”	”	”
386 ”		” ”	All dead	”	”	”

0.01% hexylresorcinol—eggs all embryonated and active.

shakings. The solutions were often unable to penetrate these masses and as a result only the peripheral eggs were killed while those in the center of the mass developed normally.

The effect of resorcinol upon ascaris eggs. The rapidity with which the different solutions kill the ascaris eggs is shown in Table I. In a 1% hexylresorcinol suspension the eggs were all killed within 5 minutes. Immediately after this immersion they appeared cleared, the cytoplasm changing from the usual deep brown to a very light yellow.

Hexylresorcinol is soluble in tap water, which is approximately neutral, to an extent of about 0.05%; ascaris eggs left as long as 15 hours in solutions of this strength developed normally to the embryonated stage. However, after 27 hours in this solution a number of the eggs were killed and the number of dead eggs increased until after 386 hours (16 days) in this solution all of the eggs were dead.

Since hexylresorcinol is more soluble in alkaline solution, in making up the 0.1% solutions, 0.2% sodium bicarbonate was added. The majority of the eggs in this solution were killed in from one to 2 hours and were all killed in 5 hours. A small percent of the eggs were killed in 30 to 45 minutes. Eggs develop normally in a 0.01% hexylresorcinol solution.

The effect of hexylresorcinol upon ascaris eggs is considerably decreased when the hexylresorcinol is dissolved in an efficient solvent. Thus a 0.1% solution of hexylresorcinol in 30% glycerine

(S.T.-37 of Sharp and Dohme) permitted normal development of the eggs. This same effect of solubility is further shown by the fact that a 1% hexylresorcinol solution made possible by the addition of 1% potassium hydroxide, did not prevent the normal development of the eggs. The increased solubility of the hexylresorcinol seems to prevent its being taken up by the ascaris eggs. This inability of the eggs to remove hexylresorcinol from solution is similar to that reported by Lamson, Ward, Brown (unpublished data) who show that the adult worm likewise is very slowly acted upon by hexylresorcinol when in solution in olive oil and glycerine in which it is quite soluble. On the other hand dilute aqueous suspensions and solutions kill them very rapidly.

TABLE II.
The Effect of Hexylresorcinol Solutions Upon the Eggs of *Ascaris suum*.
Eggs with outer albuminous coat removed.

Time in solution	0.1% Hexylresorcinol	0.05% Hexylresorcinol	S. T.-37	30% Glycerine	1% KOH
15 min.	All dead	Embryonated Active	Embryonated Active	Embryonated Active	Embryonated Active
1 hr.	” ”	”	”	”	”
5 ”	” ”	”	”	”	”
15 ”	” ”	”	”	”	”
27 ”	” ”	Few dead	Maj'y dead	”	”
48 ”	” ”	Many dead	All dead	”	”
100 ”	” ”	All dead	” ”	”	”
196 ”	” ”	” ”	” ”	”	”
386 ”	” ”	” ”	” ”	”	”

Ascaris eggs, the outside albumin coating of which had been removed by 1% potassium hydroxide, were killed more rapidly by the hexylresorcinol solutions than were the eggs with albumin coating intact (Table II). Those in the 0.1% hexylresorcinol solution were all killed within 15 minutes while those in the 0.05% solution and S.T.-37 were all killed within 100 and 48 hours respectively. The greater rapidity with which hexylresorcinol kills the ascaris eggs the albumin shell of which has been removed indicates that this layer aids materially in the resistance of ascaris eggs against chemicals. The hexylresorcinol is a protein precipitant and probably precipitates the albuminous layer to some extent, for ascaris eggs left in the hexylresorcinol solutions develop a changed albuminous outer coating as shown by the fact that it becomes much more compact and that a 1% KOH solution will no longer remove it in 48 hours, while with eggs not treated with hexylresorcinol this removal is a matter of a few minutes.

The action of resorcinol solution upon ascaris eggs is very different from that of hexylresorcinol. Resorcinol solutions of 0.1% and 1% permit normal development of the eggs, while those in a 5% solution did not develop past the one cell stage. This lack of action may be due to the great solubility of resorcinol in water. The difference in action on ascaris eggs of resorcinol and hexylresorcinol is in accord with the work of Leonard[5] who showed that the bactericidal action of resorcinol is increased by the addition of an alkyl chain and reaches its maximum activity with a 6 carbon straight chain group (n-hexylresorcinol).

Effect of hexylresorcinol upon hookworm eggs and larvae (Ancylostoma caninum).

Hookworm eggs are killed within 24 hours by a 0.05% solution of hexylresorcinol. Hookworm eggs contained in fluid feces to which was added 25 cc. of 0.05% hexylresorcinol, or 25 cc. S.T.-37 solution, all failed to develop and after 8 days were distinctly degenerated with their cytoplasm in a single dark mass. Controls to which 25 cc. of tap water had been added showed eggs in all stages of development as well as active rhabditiform and filariform larvae.

Some of the early filariform hookworm larvae placed in .05% hexylresorcinol and in S.T.-37 lost their activity within 5 minutes and were all dead in 10 minutes. Larvae in a 30% glycerine solution were likewise killed (possibly an osmotic effect) so it is not likely that the hexylresorcinol of the S.T.-37 was the only factor in their death.†

5663

Interrelation Between Secretion and Incretion.

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All attempts of different investigators to find out the interrelation between the external and internal secretion of the pancreatic gland have not been successful because the methods used, evaluation of blood sugar in the peripheral blood,[1, 2, 3] or acute pancreatico jugular transfusion experiments on anesthetized dogs[4] lack the conclusiveness of direct physiological research under natural conditions.

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[5] Leonard, V., *J. Am. Med. Assn.*, 1924, **83**, 2005.

† The hexylresorcinol for these experiments was kindly given us by Dr. Veader Leonard.

We used angiostomized dogs with pancreaticoduodenal and adrenolumbal canulas. We know the secretion curves of pancreatic juice of the dog after feeding with proteins, carbohydrates and fats.[5] We built up curves of insulin and adrenalin incretion during digestion of the same species of food.

Comparison of the curves of external and internal pancreatic secretion shows no parallelism.

After feeding with cream, which is usually followed by abundant secretion of pancreatic juice, no insulin incretion at all could be detected. After feeding with meat, after which, during the first hours, a rapid rise of external pancreatic secretion is always observed, not until the fifth hour of digestion did some internal secretion begin, lasting a short while. It is only after feeding with bread that one could find some relation between secretion and incretion of the pancreas both being represented with high curves, though there is no real similarity between them.

High curves of insulin incretion are in this case the result of abundant secretion of pancreatic juice, only because of consecutive abundant sugar formation and absorption, increasing the blood sugar level, and through it the internal pancreatic secretion. It is not a case where excretion and incretion are the result of the same stimulating agent; incretion follows another stimulating agent which is the result of the action of the external factor on the carbohydrates of the food.

Further, on comparing the curves of insulin and adrenalin incretion after different kinds of food, we see on the whole that there is mostly an antagonistic relation between them. For instance, we have a concave curve of pancreatic incretion and a convex curve of that of the suprarenal gland.

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[1] Gley and Hazard, *C. R. Soc. Biol.*, 1928, **69**, 16, 195.

[2] Jorns, *Klin. Wochenschr.*, 1929, **8**, 2319.

[3] Boldyreff, *Bull. Battle Creek San. and Hosp.*, 1929, **24**, 349.

[4] Zunz and La Barre, *Arch. Int. Physiol.*, 1930, **39**, 142; 1929, **31**.

[5] Babkin, B. P., *Die äussere Sekretion der Verdauungs drüsen.*

5664

Plasma Protein Distribution by the Modified Wu Colorimetric Method During Glucose Tolerance Tests.

J. W. CAVETT AND L. B. WINKELSTEIN. (Introduced by F. H. Scott.)

From the Departments of Physiological Chemistry and Physiology, University of Minnesota.

Wu[1] published a colorimetric method for the analysis of plasma proteins by use of a phospho-18-molybdictungstic acid (phenol) reagent. The method of fractionation and the standard used was modified by Gibson[2] and his method in essential details has been followed in this work. Six cc. of blood were removed by vena puncture, oxalated and centrifuged to obtain the clear plasma. One cc. of the plasma thus obtained was placed in a 15 cc. centrifuge tube and 2 cc. of distilled water added. To this was added 1 cc. of saturated ammonium sulphate thus bringing the solution to one-fourth saturation, precipitating out the fibrinogen. After standing 10 minutes the fibrinogen was lightly centrifuged and the fluid decanted into a dry tube. One cc. of the fluid was placed into a second 15 cc. centrifuge tube with ½ cc. of water and 1 cc. of saturated ammonium sulphate. This brought the mixture to half saturation thus precipitating out the globulins. After standing 15-20 min. the tubes were centrifuged rapidly, and the supernatant fluid decanted off. One cc. of this fluid and 2 cc. of water were pipetted into a third centrifuge tube for the albumin determination.

After all the liquid had been drained from the fibrinogen and globulin, the precipitates were dissolved in 3 cc. of water. From this point the separated fractions were treated in the same manner. To each tube was added 1 cc. of trichloracetic acid (20%) and the resulting precipitate centrifuged out. The fluid was removed, 2 cc. of distilled water blown from a pipette so as to cause a dispersion of protein and one drop of concentrated sodium hydroxide added to cause solution. The volume was brought to 9 cc.

Five cc. of standard solutions of fibrinogen, globulin and albumin (100 mg. per 100 cc.) were diluted to 9 cc. and placed in a water bath at 40°C. along with the unknowns. Clouding takes place if this is carried out at room temperature. Two cc. of phenol reagent and 3 cc. of 20% sodium carbonate were added and the tubes allowed to remain in the water bath for 10 min. They were then compared in a colorimeter with the respective standard.

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[1] Wu, H., *J. Biol. Chem.*, 1922, **12**, 33.

[2] Gibson, R. B., private communication.

Standards: The albumin and globulin used for the standards were obtained from human ascitic fluid by fractionation with ammonium sulphate and heat coagulation. The fibrinogen was obtained from beef blood in a similar manner. Sodium hydroxide was used to cause their solution. This type of standard is probably more accurate and is easier to compare than tyrosine.

Calculation of results are as follows:

Fibrinogen; $5/R = \%$ fibrinogen when standard is set at 10.
Globulin; $.50/R \times 0.4 = \%$ globulin ” ” ” ”
Albumin; $50/R = \%$ albumin ” ” ” ”

Normal protein distribution was studied on 26 individuals most of whom were medical students. The following table summarizes the results obtained:

TABLE I.

	Fibrinogen	Globulin	Albumin	Total Protein
Maximum	0.59	2.74	5.21	7.70
Minimum	0.33	1.42	3.33	5.95
Average	0.46	1.93	4.35	6.71
Standard deviation	0.06	0.28	0.42	

Figures express grams protein per 100 cc. of plasma.

The protein distribution during sugar tolerance tests as determined for 9 normal individuals. As hemoglobin is always reduced when the blood glucose increases under these conditions it was supposed that the proteins would also be diluted. However this was not the case.

The sugar tolerance curve was obtained in the usual manner. Fifty grams of glucose were given by mouth. Blood sugar was determined at the fasting level and ½, 1 and 2 hours after receiving the glucose. The protein distributions were studied at the fasting

TABLE II.

Protein	No change	Dilution	Concentration	Conc. 1 hr. Dilut. 2 hr.	Dilut. 1 hr. Conc. 2 hr.	No result
Fibrinogen	2	2	1	2	—	2
Globulin	2	2	3	1	1	
Albumin	1	5	3	—	—	
Total protein	1	4	3	1	—	

Numbers express the cases out of 9 falling into each class.

level and the 1 and 2 hour periods. A summary of the results obtained is given in Table II.

One individual showed no change in the proteins during this test. All of the others showed changes in one or all of the fractions but no correlation could be found between the protein variations and the blood sugar level, or the type of sugar curve. In 3 cases the albumin decreased and the globulin increased while in 1 case the reverse of this was true. In the other cases both proteins gave the same type of change.

Summary: The Wu method for protein distribution as modified by Gibson gives a rapid, accurate method for determination of plasma proteins. Protein is used as a standard. During glucose tolerance tests there is no relation between the protein distribution and blood sugar as is found with hemoglobin and the blood sugar.

5665

An Electrophysiological Study of the "Simple" Reflex Circuit.

LEE EDWARD TRAVIS AND HAROLD R. FOSSLER.

From the Psychopathic Hospital, Iowa City.

It is generally conceded that there are no well isolated reflex arcs. Nervous energy released in the reflex arc sense organs spreads apparently to immediately circumjacent and even to distant and perhaps to all parts of the central nervous system.* Positive evidence pertaining directly to this point has been furnished by recent experiments.[1] In the rat it has been demonstrated that the electrical energy released in the reflex arc sense organs was conducted to all parts of the cerebral cortex studied. These studies did not disclose the possible immediacy of spread of the electrical energy in the cord. The energy recorded in the cortex might have reached there by relatively sharply delimited circuits to spread subsequently to all cortical fields. It might have reached there by way of all of the afferent spino cerebral-paths. To shed further light upon this particular problem as well as upon the general problem of mass responsiveness in the central nervous system the present study was undertaken.

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* The morphological concomitant of this physiological concept is presented by Michaels, J. J., *J. Nerv. and Mental Disease,* in press.

[1] Travis, L. E., and Herren, R. Y., *J. Comp. Psychology,* 1931, in press.

The apparatus used consisted of 2 identical, non-interfering, resistance coupled amplifiers, acoustically and electrically shielded which gave identical reproduction of any impressed voltage with respect to wave form, phase, and amplitude. With 2 equally sensitive, approximately critically damped oscillograph elements the complete apparatus made possible the faithful simultaneous recording of electrical currents from varyingly separated parts of the nervous system. The recording of the instant of stimulation was accomplished by means of a specially devised vacuum tube circuit which upon the striking of a small copper plate over the tendon activated an oscillograph element. Time values of 0.001 second used for reading the reflex conduction latencies were furnished by a General Radio Type 377 low frequency oscillator. Both sets of electrodes consisted of 1 mm. apart silver wires sharpened to needle points and covered with a thin coat of insulating bakelite varnish. The ends of the needles were bared 0.5 mm. and coated with silver chloride.

The animals used were full grown white and hooded rats. The cord was prepared for electropuncture with the animal under general ether anesthetic. Experiments were conducted under light ether anesthetic. In all of these experiments the Achilles reflex of both sides was used to study electrical conduction in the cord. The rat was suspended in such a way as to permit free action of the hind extremities.

Upon striking the Achilles tendon electrical currents consistently appeared in both halves of the cord. They may or may not appear simultaneously (Fig. 1). There seemed to be no significant and consistent differences between contralateral and ipsolateral reflex

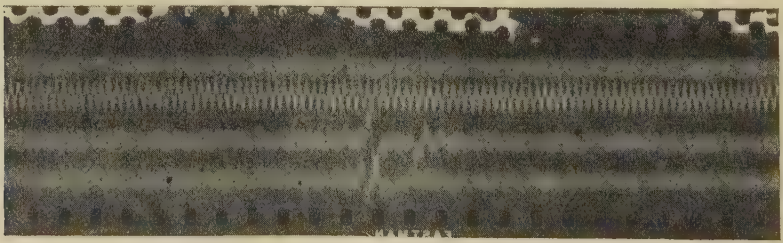

FIG. 1.

Record of electrical changes in the right and the left side of the cord as a direct result of Achilles reflex stimulation. Reading from above downward the first record is of the instant of striking the Achilles tendon, the second of time in 0.001 second interval values, the third of electrical changes in the left side of the cord, and the fourth of electrical changes in the right side of the cord. The conduction latency for the left side of the cord was read as 0.0025 second and that for the right side of the cord was read as 0.0015 second.

conduction latencies (Table I). Furthermore according to our readings the distance of the point of insertion of the electrodes from the spinal cord origin of the sciatic nerve did not make any consistent difference in the latencies.

The data indicate that the electrical energy conducted to the central nervous system along the sciatic nerve is relayed immediately upon its arrival to both halves of the cord.

For the rat Travis and Herren[1] determined the Achilles reflex response latency (the time elapsing between the striking of the Achilles tendon and the appearance of electrical changes in the gastrocnemus muscle) to be 6.7 sigma. The Achilles reflex arc consists of at least the receptor, an afferent path, a synapse in the cord, an efferent path, muscle end-plates, and the effector. The conducting circuit dealt with in the majority of the animals in the present study consisted of at least the receptor, an afferent path to the cord, and an afferent path in the cord. This latter afferent path may be the spinal cord portion of the afferent component of the sciatic nerve. It may be a separate central afferent path. Comparing 6.7 sigma with 2.5 sigma (the time elapsing between the striking of the Achilles tendon and the appearance of electrical

TABLE I.
Reflex Conduction Latencies in the Spinal Cord.

Rat	Position of Electrodes in Relation to Spinal Cord Origin of Sciatic Nerve	Latency in 0.001 second			
		Contralateral		Ipsolateral	
		Mean*	Range	Mean*	Range
1	One segment posterior			2.9	2.5—3.5
2	Same ”	2.3	2.0—2.5	2.0	2.0—2.0
3	” ”	2.1	1.5—2.5	2.7	2.5—3.0
4	” ”	2.3	2.0—3.0		
5	” ”	2.2	2.0—2.5	2.2	1.5—2.5
6	” ”			2.0	2.0—2.0
7	” ”			3.3	3.0—4.0
8	One segment anterior	3.0	3.0—3.0	2.5	2.5—2.5
9	” ” ”	1.8	1.5—2.5	2.0	2.0—2.0
10	” ” ”	1.8	1.5—2.0	2.9	2.0—3.5
11	” ” ”	2.5	2.0—3.0		
12	” ” ”	2.6	2.5—3.0	2.7	2.5—3.0
13	” ” ”	3.0	2.0—4.0	2.2	2.0—3.0
14	” ” ”			2.5	2.0—3.5
15	Two segments ”	4.1	4.0—4.5	3.0	3.0—3.0
16	” ” ”	2.5	2.5—2.5		
17	Four ” ”	3.0	3.0—3.0		
18	” ” ”	2.4	2.0—2.5	2.5	2.0—3.0
19	” ” ”	2.6	2.0—3.0	2.9	2.5—3.0
	Mean	2.5		2.5	

* Average from 3 to 10 readings.

changes in the cord) we note that the latter value represents slightly more than one-third of the former. Although anatomically we placed the electrodes at a point about midway in the Achilles reflex arc our mean latency value is much less than half the mean Achilles reflex response latency value. Electrophysiologically considered, the segment of the cord containing the assumed central portion of the Achilles reflex arc is not the half-way point in the arc. Either more structures or structures with higher resistances to the passage of the electrical changes we are studying must lie between this commonly conceded mid-point and the responding muscle tissue than between the sense organs and such a cord center.

The relatively great variations in latencies found in not only different rats but also in the same rat, although not readily understandable, may be partially explained in that from rat to rat and from record to record in the same rat the depth of anaesthesia varied.

In its possible effect upon the dynamic relationships existing between higher and lower neural levels and upon the conducting circuits mediating between these levels this implied irradiation of the reflex influence would appear to be highly significant.

5666

"Nucleoprotein" and Non-Protein Substance Isolated from the Gonococcus. I. Preparation.*

ALDEN K. BOOR AND C. PHILLIP MILLER, JR.

From the Department of Medicine, University of Chicago.

The preparations described below were made from 12 to 18 hour cultures of gonococci grown on an agar medium[1] containing a tryptic digest of egg-white from which the heat-coagulable proteins had been removed. The organisms were taken up in saline, centrifuged, washed and again centrifuged until well packed. They were then extracted by suspending each cubic centimeter of packed, moist organisms in 200 cc. of N/100 NaOH and allowing the suspension to stand over night in the refrigerator. After removal of the bacterial debris by centrifugation the clear supernatant liquid was acidified with acetic acid to maximum precipitation.

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* This study was made possible by a research grant from the Public Health Institute of Chicago.

[1] Miller, C. P., and Castles, R., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 123.

The precipitate comprising the so-called bacterial "nucleoprotein" was separated from the supernatant liquid (the subsequent treatment of which is described below) by centrifugation, redissolved in N/100 NaOH, and reprecipitated with dilute acetic acid. After 4 to 6 repetitions of this procedure, the final solution of the "nucleoprotein" in N/100 NaOH was dialysed against distilled water and the neutral suspension evaporated to dryness at 40°-60°C. in an air current. In the dry state it has been kept at room temperature for several months without evidence of deterioration. The usual reactions of proteins were all positive: biuret, Millon, Xanthroproteic, Hopkins-Cole, sulpho-salicylic and phosphotungstic acid.

The non-protein fraction was obtained from the supernatant fluid of the first acetic acid precipitation. This liquid was evaporated to small bulk (about 10 cc.) heated in a boiling water bath for 15 minutes, filtered from coagulated protein and then treated with about 10 volumes of 95% ethyl alcohol. After standing long enough to insure complete denaturation of any denaturable proteins the precipitate was removed by centrifugation, dissolved in water, filtered, reduced to small bulk by evaporation and again treated with alcohol. After 2 or 3 such reprecipitations the aqueous solution was allowed to stand over night in the refrigerator and dialyzed against distilled water. The neutral solution was then evaporated to dryness in the same manner as the protein fraction. Like the latter it has been found to keep satisfactorily in the dry state at room temperature.

This non-protein fraction gave a positive Molisch test. The Benedict-Fehling reaction was negative before and positive after acid hydrolysis. All of the tests for protein enumerated above were negative. It appears, therefore, to consist largely of carbohydrate, presumably a polysaccharide.

The unexpected non-specific reactions described in the third paper of this series[2] aroused the suspicion that the repeated treatment with N/100 NaOH might have been so heroic as to have denatured the isolated substances sufficiently to alter their antigenic properties. This possibility was ruled out by checking an adequate number of precipitin reactions with preparations made by extracting washed gonococci (whose reaction was pH = 6.4) with distilled water. Water, however, was so much less efficient than sodium hydroxide as an extracting agent that its use was abandoned.

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[2] Boor, A. K., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1050.

5667

"Nucleoprotein" and Non-Protein Substances Isolated from the Gonococcus. II. Immunological Reactions with Anti-Gonococcus Serum.

ALDEN K. BOOR AND C. PHILLIP MILLER, JR.

From the Department of Medicine, University of Chicago.

Anti-gonococcus sera were prepared by injecting rabbits intravenously with washed, live organisms from 18-hour cultures grown on an agar medium containing a tryptic digest of egg-white.[1] The 5 individual strains employed included recently isolated ones and old stock strains which had undergone some 400 transplantations in this laboratory. The rabbits were obtained from a special stock of snuffle-free animals. Sera against a mixture of stock strains and against the protein fraction described in the preceding paper[2] were also prepared.

No antiserum against the non-protein fraction was obtained.

Precipitin reactions were run with each immune serum using as precipitinogens the protein and non-protein fractions prepared from each of the 5 strains. Ring tests were made by stratifying the precipitinogen, in progressive 10-fold dilution, over the serum (diluted with 2 parts of saline) in small tubes of 4 mm. inside diameter. Readings were made after standing at room temperature for one hour.

The table gives a sample of the results of the precipitin tests on the various protein and non-protein fractions, using rabbit immune sera prepared by the injection of (a) whole organisms of one strain, (b) the "nucleoprotein" of that strain, and (c) whole organisms of a mixture of strains. The titers of the "nucleoproteins" were somewhat higher than those of the non-protein fractions.

It is noteworthy that the anti-nucleoprotein serum gave positive tests with the non-protein substances. This probably indicates the

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[1] Miller, C. P., and Castles, R., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 123.

[2] Boor, A. K., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1046.

TABLE I. Titres of Representative Precipitin Reactions.

Precipitinogen	Immune Sera Prepared Against:		
	Whole organisms Strain 1	"Nucleoprotein" Strain 1	Whole organisms Many strains
"Nucleoprotein" strain 1	+++ ++	+++ +	+++ +++
" " 3	+++ +	+++ +	+++ +
" " 5	+++ ++	+++ ++	+++ ++
" " M6B2	+++ ++	+++ +	+++ ++
" " 10	+++ +++	+++ ++	+++ +
Non-protein fraction strain 1	+++	+++	+++ +
" " " 3	+++ +	+++ +	+++ +
" " " 5	+++ ++	+++	+++ +
" " " M6B2	+++ ++	+++	+++ +
" " " 10	+++	+++ +	+++ +

The plus marks (+) indicate the dilutions as multiples of 10; thus: +++ = 1:1,000; +++ + = 1:10,000, etc.

presence in the "nucleoprotein" of the reactive substance of the non-protein fraction, which we believe to be a polysaccharide. In view of the process of purification it may be assumed that the polysaccharide is present as part of the protein aggregate. Another possibility which has not been eliminated is the incomplete removal of the last traces of protein from the non-protein fraction, although it gives no chemical tests for proteins.

No evidence of strain specificity was observed among the 5 strains from which non-protein fractions have been prepared. Casper[3] has reported (in a paper which has but recently come to our attention, although it was published last November) the isolation from gonococci of a non-protein substance, containing polysaccharide, which was type specific for each of 2 types which he was able to differentiate on the basis of agglutination reactions. He made his preparations from bile solutions of gonococci and employed them in a study of the cutaneous reactions of patients suffering from gonorrhoea.

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[3] *Klin. Wochsch.*, 1930, **9**, 2154.

5668

"Nucleoprotein" and Non-Protein Substance Isolated from the Gonococcus. III. Immunological Reactions with Anti-Meningococcus and Anti-Pneumococcus Sera.

ALDEN K. BOOR AND C. PHILLIP MILLER, JR.

From the Department of Medicine, University of Chicago.

Using the "nucleoprotein" and the non-protein fractions prepared from 5 strains of gonococcus[1] positive precipitin reactions were obtained with anti-meningococcus and anti-pneumococcus sera, using the technique described in the preceding paper.[2] The sera were obtained from 3 commercial firms and from the laboratories of the New York State Board of Health. The table shows representative precipitin titres for one of each kind of serum employed.

TABLE I.
Titres of Precipitin Reactions.

Precipitinogen	Immune Sera			
	Anti-pneumococcus			Anti-meningococcus
	Type I	Type II	Type III	
"Nucleoprotein" strain 1	+++	+++	++++	+++ ++++
" " 3	+++	+++	++++	+++ +++
Non-protein fraction strain 1	+++ ++	++++	+++ +++	+++ ++++
" " " 3	++++	++++	+++ +++	+++ ++++

The plus marks (+) indicate the dilutions as multiples of 10, thus: +++ = 1:1,000; +++ + = 1:10,000, etc.

The cross reactions with the anti-meningococcus sera were not unexpected, but those with the anti-pneumococcus sera were quite surprising. It should be noted that whereas our anti-gonococcus sera were precipitated by gonococcus "nucleoprotein" in higher dilution than by the non-protein fraction, the ratio was reversed in the case of the anti-pneumococcus sera.

Precipitin reactions qualitatively similar to those given in the table were obtained with filtrates of broth cultures which had been submitted to no chemical treatment. The titres of such filtrates cannot be estimated because the concentration of antigen has not yet been determined.

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[1] Boor, A. K., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1046.

[2] Boor, A. K., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1048.

5669

The Invariants of the Electrocardiogram.*

ERNEST BLOOMFIELD ZEISLER. (Introduced by Louis N. Katz.)

From the Heart Station, Michael Reese Hospital, Chicago.

By recording electrocardiograms with many different leads it is possible to find those characteristics which are common to all the curves, that is, which are independent of the particular lead employed. Such characteristics are called the *Invariants* of the electrocardiogram and correspond to the essential properties of the electrical changes created during the heart action. It is found that these invariants are (1) recurring cycles of 3 complexes each, and (2) the time-intervals between the complexes and of the span of the individual complexes.

5670

Electrocardiographic Studies in Subtotal Atelectasis and Pneumectomy in Dogs.

WAYNE GORDON AND W. E. ADAMS. (Introduced by E. Andrews.)

From the Departments of Medicine and Surgery, University of Chicago.

Changes in the shape of the complexes of the electrocardiogram have been observed in pathologic intrathoracic conditions not primarily cardiac. These conditions include pericarditis,[1] pericardial effusion without demonstrable inflammation,[2] and lobar pneumonia.[3] Bettman and Priest[4] described the electrocardiograms of patients before and after chest operations, including in their series 7 patients on whom extrapleural thoracoplasty was performed. One of the 7 developed the so-called "coronary type" T-wave and 3 others diminution in the amplitude of the QRS complex. These findings suggested a study of the electrocardiograms of dogs following the production of atelectasis or the removal of one or more lobes of lung. The operations were done as described elsewhere by Adams[5] and the amount of functioning lung tissue calculated according to his table.

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* Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

[1] Porte, Daniel, and Pardee, H. E. B., *Am. Heart J.*, 1929, **4**, 584.

[2] Scott, R. W., Feil, H. S., and Katz, L. N., *Am. Heart J.*, 1929, **5**, 68.

[3] Shearer, M. G., *Am. Heart J.*, 1930, **5**, 801.

[4] Bettman, R. B., and Priest, W. S., *Am. Heart J.*, 1930, **5**, 366.

Nine dogs were studied in which collapse of one or more of the pulmonary lobes had taken place more than a month previously. In this group 2 had sinoauricular block and 4 had inversion of the T-waves in leads 2 and 3. One had depression of the ST segment below the isoelectric line with inversion of T in leads 2 and 3. QRS was of very low amplitude in all leads in one dog. This finding was confirmed in repeated tracings. The dogs in this series had from 43% to 57% of functional lung tissue.

On one dog a tracing was taken the next day after the collapse of the left upper lobe. The entire right lung had been collapsed more than a month previously, so that at the time the tracing was taken only 28% of the original lung tissue remained functional. This dog had a notched but upright T-wave in all leads. The rate averaged 60 per minute. There were a few points in the tracing where the intervals between beats were exactly half the adjacent intervals and it was felt that this dog probably had a marked sinoauricular block.

One of the dogs was observed later during the collapse of the left lower lobe 11 days after the first tracing was taken. An electrocardiogram taken soon after the onset of dyspnea, before atelectasis had progressed far as observed under the fluoroscope, contained a depressed ST interval as had been previously observed. Three hours later, when fluoroscopic evidence indicated the atelectasis to be progressed much further, the ST interval began 1.5 mm. above the isoelectric level, was conves downward to the isoelectric level, and ended in an upright T-wave. An inverted T-wave preceding the upright peak which had been present 3 hours previously had completely disappeared. Electrocardiographic tracings taken 24 hours after the onset of dyspnea showed no further change. Following this collapse the left upper lobe was the only functional lung tissue in this dog.

Three dogs were examined in which there had been atelectasis followed by surgical removal of one or more atelectatic lobes. Of these one had an inverted T-wave in leads 2 and 3, two alterations in the shape and level of the ST segment, and one sinoauricular block. The amount of functional lung tissue was 33 to 57%.

All the dogs seen in this experiment had very well-marked sinus arrhythmia. The sinoauricular block type of curve occurring in 4 of the 13 dogs with part of the lung out of function may be considered to differ from the sinus arrhythmia in these cases only in degree. Both types of rhythm are seen in normal dogs, although in our experience they do not occur so frequently. We consider these arrhythmias to be due to vagal action. The inversion of the T-wave is of questionable significance when the frequency of this phenomenon in the dog is considered. The ST changes are slight, but are sufficiently unusual to suggest further investigation, including control electrocardiograms preceding operation. We do not feel that we have sufficient data to explain the ST changes at the present time.

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[5] Adams, W. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**.

5671

Rate of Elimination of Certain Toxic Substances from the Blood.*

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From the Department of Surgery, University of Chicago.

In certain diseases it is believed that the symptoms are due to a toxin which circulates in appreciable amounts in the blood stream. Efforts have been made to demonstrate this substance in the blood of diseased men and animals by chemical analysis, or by the injection of their whole blood, blood serum or blood extracts into healthy animals. These injections have been given intravenously or intraperitoneally. It was believed that if the individual was poisoned by such a toxin, the injection of his blood or blood serum into healthy animals would produce toxic symptoms in them. The negative results in these experiments have been considered proof of the absence of such an intoxication.

It seemed to us, however, that toxic substances might be so rapidly removed from or inactivated in the blood that although an individual was suffering from a profound poisoning, the substance might not be demonstrable in the blood stream.

The following experiment was devised to test this assumption. Dogs were matched as for size and the compatibility of their bloods and were anesthetized with sodium barbital intravenously (200 to 250 mg. per kg.) or by stomach tube (250 to 300 mg. per kg.). They were then prepared for the typical carotid-artery-jugular vein

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

crossing of circulations. In addition the transfer of blood was recorded and kept equal by means of the Montgomery and Lipscomb volume flow apparatus.[1] Heparin (30 mg. per kg.) was used as an anticoagulant and the blood pressure was taken from the femoral artery. The dogs were labeled A and B. The drug to be tested was injected into the femoral vein of dog A and was allowed to circulate for varying intervals of time, after which the 2 circulations were crossed. Dog B was observed for changes characteristic of the drug.

To date only strychnine has been tested under control of blood transfer. It was found in confirmation of the chemical studies of Weiss and Hatcher[2] that this drug was rapidly removed from or inactivated in the blood stream. Twice the lethal dose of strychnine (1.5 mg. per kg.) for an animal under anesthesia was injected into dog A, which promptly developed severe convulsions. Within 3 minutes so much of the drug was removed that crossing of the circulations caused no convulsions, although the bloods were continued in cross circulation for over 30 minutes at the rate of 180 to 220 cc. per minute. In another experiment when the bloods were crossed 1.5 minutes after injection of a similar dose dog B developed convulsions 12 minutes after the crossing of the circulations.

Some experiments done without the control of the transfer of blood indicate that, as measured by cross circulation studies, other drugs also are rapidly eliminated. Tests with these and the toxic substances formed in or found in animals (histamine, guanidine, etc.) are now being made.

This work seems to show that it may be possible for a man or an animal to be profoundly poisoned by a substance which cannot be demonstrated in the blood by transfusion experiments and that therefore the inability to produce toxic symptoms in healthy animals by this means does not necessarily rule out the existence of such an intoxication in the diseased animals.

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[1] Montgomery, M. Laurence, and Lipscomb, T. H., *Am. J. Physiol.*, 1929, **90**, 454.

[2] Weiss, S., and Hatcher, R. A., *J. Pharm. and Exp. Therap.*, 1922, **19**, 419.

5672

A Method for the Determination of Enterokinase.

ROBERT W. BATES. (Introduced by F. C. Koch.)

From the Department of Physiological Chemistry, University of Chicago.

Any method of assay for enterokinase is indirect, and consists in a determination of the amount of trypsin which the enterokinase can produce from a given amount of trypsinogen. The amount of trypsin is determined by means of its digestive action on some suitable protein substrate. The methods used by Waldschmidt-Leitz,[1] and Linderstrom-Lang and Steenberg[2] consist in a determination of that amount of kinase which will just give maximal activity to a given quantity of trypsinogen or only one-half of the maximal activity in a given period of time, usually 30 minutes.

Such methods of assay involve all of the variables and possible errors of a trypsin assay plus a great number involved with the activation process. A brief study has shown that many gross errors are easily incurred during activation. Activation was found to occur in the presence of the alkaline protein substrate so that any method of partial activation before addition of the protein substrate is complicated by the additional amount of activation which occurs subsequently. The amount of kinase which will impart maximal activity to a given quantity of trypsinogen is not a clean-cut quantity. As one adds increasing amounts of enterokinase to a constant quantity of trypsinogen a maximum value is almost never reached. It is reached very gradually so that a small error at any point in the determination would be magnified in the final estimation.

In assaying enzymes it is customary to have present an excess of substrate. Assuming the action of enterokinase to be that of an enzyme and since activation occurs during digestion and at a slower rate than at the neutral pH usually used for activation, it seemed logical to attempt to assay by using an excess of trypsinogen with no activation with the added enterokinase, other than that occurring simultaneously with the digestion. Such a system is obviously complex but no more so than when activation is carried out as a separate step. The results, however, show very simple relationships and the assay of a sample of enterokinase is easily accomplished in less time than that of a sample of trypsinogen since the period of activation is omitted.

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[1] Waldschmidt-Leitz, E., *Z. Physiol. Chem.*, 1924, **132**, 181.

[2] Linderstrom-Lang, K., and Steenberg, E. M., *Compt. rend. trav. Lab. Carlsberg*, 1929, **17**, 1.

The materials used for the enzyme solutions were dry trypsin-free trypsinogen powders and acetone defatted duodenal mucosa. The protein substrate was 6% casein in 0.4% sodium carbonate. The amount of digestion was determined by observing the change in refractive index of isoelectric filtrates of the substrate at once and after 4 hours digestion at 37°C.

It has been found that below a change of R.I. of 5 scale divisions on a Bausch and Lomb immersion refractometer, the change is almost directly proportional to the concentration of the trypsin. By using trypsinogen equivalent to 4 times the amount of trypsin which will cause a refractive index change of 5, and adding variable amounts of enterokinase, it was found when the total resulting change in refractive index is less than 5 scale divisions, that this change is directly proportional to the concentration of enterokinase added.

$$\Delta \text{ R.I.} = k \times \text{mg. enterokinase.}$$

Also, if both trypsinogen and kinase are varied the product of the two, times a constant, gives the change of refractive index:

$$\Delta \text{ R.I.} = k \times \text{mg. trypsinogen} \times \text{mg. enterokinase.}$$

For practical work, however, it is simpler to plot a curve using a constant amount of trypsinogen and variable amounts of a known enterokinase preparation and to read subsequent values from this curve. This method is not free from errors, but is rapid and gives values more easily reproduced than other proposed methods.

5673

Action of Morphine on the Intestine in Peritonitis.

C. J. WILEN AND C. A. DRAGSTEDT.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

For many years the administration of morphine in peritonitis has been advocated by many surgical authorities. It has been advocated not only for the relief of pain and distress, but for the purpose of putting the intestine at rest in order to restrict or prevent the spreading of the infection that intestinal motility might produce. The well established observation that morphine tends to produce constipation was interpreted to mean that morphine did stop intestinal motility.

Plant and Miller[1] reported observations that both in the dog and man, morphine produces an increase in intestinal tone and an increased frequency of contractions, contradicting the current conception that morphine decreases intestinal activity.

Because of the relatively established position of the use of morphine in the treatment of peritonitis it was of interest to determine if the existence of a peritonitis modified the type of response to the usual therapeutic doses.

Peritonitis was produced in 10 dogs by the injection of fecal emulsions or by allowing a segment of intestine to drain into the peritoneal cavity. Intestinal motility was recorded from a balloon placed either in a Thiery-Vella fistula or in the intestinal tract. In every instance the subcutaneous injection of 10 mg. of morphine sulphate produced an augmentation of intestinal activity which was not antagonized by atropine sulphate, except in 2 instances in which during the 12 hours preceding death, spontaneous movements were absent and the intestine failed to respond to either morphine or physostigmine. Observations on 4 cases of peritonitis at the Cook County Hospital have all indicated increased intestinal motility after morphine sulphate, as determined by auscultation of the abdomen with a stethoscope before and after its administration. If it can be assumed that the general use of morphine in the treatment of peritonitis warrants the statement that its administration is beneficial, the interpretation of its beneficial action must be on some other basis than that of abolishing intestinal activity and putting the intestine at rest.

5674

Ultraviolet Point Radiation in the Production of Arrythmias in the Heart of the Chick Embryo.*

MARIE A. HINRICHS and GEORGE WARRICK.

From the Department of Physiology, University of Chicago.

By means of apparatus previously described,[1] a point source of radiation could be directed on the desired spot on the developing

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[1] Plant, O. H., and Miller, G. H., *J. Pharm. and Exp. Therap.*, 1926, **27**, 361.

* Supported in part by a grant from the Rockefeller Foundation to the University of Chicago, and in part by a grant from the Radiation Fund of the National Research Council.

[1] Hinrichs, M. A., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 354.

embryonic heart and its effect on the rhythm noted. Studies were made on embryos varying in age from 2 to 20 days, and the following results obtained: Short exposures of the sinus region of the heart produced an increase in rate of beat within 30 seconds after the exposure was begun. A long-continued exposure slowed the rate. Exposure on the ventral aspect of either auricle (at a point of visible condensation of tissue) also produced a marked increase. Exposure of the ventricle did not change the rate of beat except when the point of the quartz rod was placed on the interventricular septum at the base of the auricles. A slight increase was then obtained in hearts whose rate had already been slowed.

Also, in young hearts (2 to 6 days old), it was possible to obtain complete reversal of the direction of beat by a preliminary slowing brought about by lowering the temperature, and then radiating the ventricle or bulbus region.

The normal sequence of the heart cycle may be changed by radiating the sinus or auricle. When a heart has already slowed its rate of beat following continued exposure, a 1:1 auricular-ventricular rhythm may be gradually changed to a 2:1, and then a 3:1, and so on. (A 5:1 ratio was maintained in one heart for several minutes before a further change was produced.) In some cases it was possible to obliterate the ventricular beat entirely, while in others the sinus alone remained beating. Partial or complete heart block can be produced.

Ectopic beats of auricular origin have been obtained by local stimulation of the auricle.

Twenty-six hearts were studied in this way, and in every case it was possible to modify the rate of beat or its rhythm, depending on the dosage used, and on the location of the point of the quartz rod on the heart. Tachycardia and bradycardia were studied in 11 cases, arrhythmias were produced in 10 hearts, while in 5 young embryos it was possible to reverse the direction of beat completely.

Heart rhythms may thus be varied by producing a local change in physiological activity, and the effect may or may not be transmitted to other regions of the heart depending on the condition of (a) the region radiated, (b) the transmitting tissue, and (c) the remaining heart tissue.

5675

Ultraviolet Point Radiation in Production of Developmental Abnormalities in the Chick Embryo.*

MARIE A. HINRICHS.

From the Department of Physiology, University of Chicago.

By means of the apparatus described by Hinrichs, ultraviolet point radiation was used to locally modify the development of chick embryos.[1] At various intervals during incubation, eggs were opened,[2] and the desired region exposed for a given period of time, after which the shell was again sealed shut, and the incubation continued.

Most of the present experiments were made on the developing eye, and results were obtained as follows: (a) inhibition of the rate of development as compared with the unradiated eye, (b) killing of lens tissue, (c) stimulation of rate of pigment formation in the optic cup. The type of effect obtained depended on the dosage and the age of the embryo.

Exposures of the developing brain resulted as follows: short exposure of the forebrain of a 2-day embryo produced a lateral overgrowth on the exposed side. No such effect was obtained in the hindbrain. Longer exposures produced a coagulation of brain tissue. Exposure of the median posterior region of a 2-day embryo caused degeneration of that region.

Even moderate exposures of the heart (40 sec.) produced a slowing of the rate of beat in a 2-day chick, and on subsequent incubation, a loss of contractility of the exposed region.

An interesting condition was brought about following a 45-second exposure of the base of the aortic arches. On the exposed side, the arches atrophied as did also the tissues and organs supplied by them, while on the unradiated side, the arches and vessels were distended, appeared larger than normal, and the organs on that side of the body were completely normal in their development.

It was found that the developing tail and limb buds were relatively non-susceptible to moderate doses (45-60 sec.), while a 90-second exposure produced a coagulation of tissue in the hind limb of a 2-day chick.

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*Supported in part by a grant from the Rockefeller Foundation to the University of Chicago, and in part by a grant from the Radiation Fund of the National Research Council.

[1] Hinrichs, M. A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 354.

[2] Hinrichs, M. A., *J. Exp. Zool.*, 1927, **47**, 309.

Eighty-five exposures were made, and of these 52% died as the result of either too high a dosage of radiation, of mechanical injuries produced during the operation, or of incomplete closure of the shell so that bacterial infection proved fatal. Forty-one cases remained to undergo partial development. Of these, 5 showed no developmental changes in radiated regions because of too short a period of exposure. Modifications in the other 36 cases were brought about as follows: 43% in the heart and aortic arches, 48% in the eye and forebrain regions, and 9% in tail and limb buds.

While in these experiments mortality is high, due in part to factors other than radiation,[2] nevertheless the method is one well adapted for the localization of injuries during development in such forms as the chick. With the maintenance of a constant dosage, it could be adapted for testing the relative susceptibility of various regions of the embryo at different stages in the developmental period. Certainly, in these experiments, the parts of the nervous system tested, as well as those of the circulatory system, were far more susceptible than such regions as the developing limb buds at a corresponding age.

5676

Relative Toxicity of Antiseptics on Bacteria and Tissues in Cultures.*

RALPH BUCHSBAUM AND WILLIAM BLOOM.

From the Department of Anatomy, University of Chicago.

Lambert,[1] Lambert and Meyer,[2] and German[3] believe that an ideal antiseptic is one which kills the bacteria without harming the tissue cells and they have accordingly devised methods of comparing the toxic effects of antiseptics upon bacteria and tissue cells *in vitro*.

Lambert after exposing fragments of connective tissue for several minutes to a saline suspension of *Staphylococcus aureus* transferred them into various concentrations of mercuric chloride, po-

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* This investigation was aided by a grant from the Eli Lilly Co.

[1] Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

[2] Lambert, R. A., and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.

[3] German, W. J., *Arch. Surg.*, 1929, **18**, 1920.

tassium mercuric iodide, potassium cyanide, Dakin's solution, iodine, phenol, tricresol, argyrol, hydrogen peroxide, glycerol, or alcohol. After one hour the fragments were transferred to physiological salt solution where they remained from a few minutes to half an hour until explanted. Although most of the antiseptics killed the tissue cells at concentrations that did not kill the bacteria, a good growth of cells was observed after exposure for one hour to a 1:2,000 iodine solution, a strength sufficient to sterilize the tissue completely in most instances.

Lambert and Meyer placed rabbit spleen fragments in a *Staphylococcus aureus* suspension for one minute, and then for 20 minutes in various concentrations of alcohol, iodine, mercuric chloride, mercurochrome, acriflavin, protargol, albargin, gentian violet, neosalvarsan, or hexylresorcinol. The fragments were then washed twice in salt solution. The best results were obtained with iodine, mercuric chloride, and neosalvarsan. It is possible that in these cultures the cells may have come from the center of the explant where they were partially protected. In a second series they added the antiseptics directly to the culture medium. In these cultures higher dilutions were used, and neosalvarsan alone was less toxic for cells than bacteria, i. e. inhibiting bacteria in dilutions of 1:30,000 to 1:40,000 while cells were only slightly damaged by these dilutions. They concluded that iodine and mercuric chloride are nearer the ideal than some of the newer preparations.

German placed bits of skin of chick embryos (6 to 9 days old) in various dilutions of gentian violet, acriflavin, potassium mercuric iodide, mercurochrome or picric acid for one and 5 minutes, after which they were washed in Locke's solution and mounted as tissue cultures in a medium of plasma and embryonic extract. To determine the lethal points of the bacteria (*Streptococcus hemolyticus, Staphylococcus aureus,* and *Bacterium coli*) he placed bits of muscle and fascia in broth cultures of the bacteria, and then transferred the tissues to the antiseptic solutions. After one and 5 minutes they were planted on agar plates. He believed the "efficiency" of an antiseptic to be directly proportional to its bacteriostatic effect and inversely to its harmful action on the tissues. He found maximal "efficiency indexes" (product of per cent of tissue cultures showing growth by per cent of bacterial cultures showing inhibition) at the following concentrations: gentian violet 1:1600, efficiency of 0.03; acriflavin 1:800, efficiency 0.1914; potassium mercuric iodide 1:50 to 1:1600, efficiency 0.0; mercurochrome 1:400, efficiency 0.0132; and picric acid 1:50, efficiency 0.0975. German concluded that these results show how far from perfect ("efficiency" of 1.00) the antiseptics are, but added that these are only rough estimates because of the variability of the results. However, it would appear that German's "efficiency indexes" indicate a degree of precision which is in excess of that warranted by his data. Further, the same index may be obtained from a variety of data because it represents the product of 2 variable percentages.

In our study of certain antiseptics we attempted: (a) to develop a method that would render the figures more quantitative and standard than seems to be possible with the methods described above; (b) after some preliminary experiments performed with a similar technique in this laboratory by Dr. R. Herrick, to compare the new antiseptics merthiolate (sodium-ethyl-mercurithiosalicylate) and metaphen (4-nitro-3, 5-biacetoxy-mercuri-2-cresol) with phenol by means of this technique; and (c) to determine whether the tissue culture technique could be simplified by substitution of some antiseptic for at least part of the present aseptic technique.

Experiments were conducted using: (1) cultures of spindle cells cultivated *in vitro* for several months until all of the cells were morphologically indistinguishable from one another ("pure" strain), and (2) mixed cultures derived from the second transplant of periosteum of 12-day embryonic chicks. Although the "pure" cultures gave more precise results, the second-transplant cultures gave almost as consistent results and had the advantage of being more convenient for a standard and easily reproducible test. The culture medium consisted of 2 parts of chicken plasma, one part of embryonic chick extract, and one part of various strengths of the antiseptic dissolved in Tyrode's solution.

A laboratory strain of *Staphylococcus aureus* isolated from an infected human jaw was used as the contaminating organism. A 24 hour colony from an agar plate was inoculated into 3 cc. of beef broth. The 24 hour broth culture was centrifuged and the bacteria taken up in 5 cc. of Tyrode's solution; one part of this suspension being added to 15 parts of embryonic extract. As great differences in the concentration of the bacteria were found to be a cause of variable results, it was found necessary and satisfactory to follow this method.

Five types of cultures were made: (1) antiseptic and bacteria; (2) antiseptic and tissue; (3) antiseptic, bacteria, and tissue; (4) bacteria; (5) tissue. The cultures were made with various concentrations of the antiseptics and then observed for bacterial and tissue growth after 24 and 48 hours. These time intervals were used since antiseptics are frequently allowed to remain in contact with the tissue cells for extended periods of time. At least 4 cultures were made with each dilution of each of the 5 types of cultures and these generally showed uniform results. Great care in technique is necessary in such experiments because of the small volumes used. The condensed results are shown in the accompanying table.

TABLE I.

Showing Relative Toxicity of Antiseptics on *S. Aureus* and Embryonic Chick Periosteal Cells.

Antiseptic	Dilutions used	Greatest dilution that killed the bacterium used.* $=(A_b)$	Greatest concentration in which cells show approximately normal growth. $=(A_c)$	$\frac{(A_b)}{(A_c)}$
Phenol	1:1, 1:2, 1:3 1:4, 1:5, 1:6 1:7, 1:8, 1:9 1:10 ×100	1:200	1:900	0.2
Iodine	1:2, 1:4, 1:6 1:8, 1:12, 1:15 ×1000	1:2000†	1:4000	0.5
Mercurochrome	1:4, 1:8, 1:16, 1:20, 1:24 1:28, 1:32 ×1000	1:16,000	1:32,000	0.5
Metaphen	1:20, 1:40 1:80, 1:100 1:120, 1:140 1:160, 1:180 ×1000	1:100,000	1:180,000	0.6
Merthiolate	1:20, 1:40 1:80, 1:100 1:120, 1:140 1:160, 1:180 ×1000	1:140,000	1:160,000	0.9

* The viability of the bacteria after exposure to the antiseptics was controlled by inoculating the tissue cultures into beef broth.

† Inhibited bacteria somewhat. A more concentrated solution could not be obtained without using potassium iodide.

Three kinds of results from cultures of antiseptic, bacteria, and tissue (type 3) require separate mention: (a) In concentrations that killed the bacteria in bacterial cultures (type 1), the bacteria in type 3 cultures were killed except immediately surrounding the tissue, where they grew profusely even though the explants were previously bathed for 15 minutes in the corresponding concentration of antiseptic. (b) In type 3 cultures with concentrations of antiseptic allowing bacterial but inhibiting cell growth, the bacteria grew throughout the medium, but most abundantly about the tissue. (c) Again, in type 3 cultures, with concentrations of antiseptic allowing both bacteria and cells to grow, the bacteria soon overran the entire medium and the cells died.

An antiseptic that would kill the bacteria at concentrations that would not harm the cells would have a ratio $[A_b]/[A_c]$ (an index of relative toxicity) of 1.0 or greater. Although merthiolate is the least toxic of the group as tested by us with this method, it is not an "ideal" antiseptic.

The technique here described makes it possible to obtain rather consistent results on the relative toxicity of antiseptics on bacteria and living tissues, especially if some standard antiseptic is run as a check on the method. In stating the toxicity index, the organism used should be named, as is done in the case of the phenol-coefficient, as described by Reddish,[4] and also the tissue. If other types of bacteria are used, it is possible that the method here described or some modification of it will produce an index that will be more useful than the present phenol-coefficient, which does not consider the effects of the antiseptic upon the tissue cells.

5677

Effect of Hypertonic Solutions on Cerebrospinal Fluid Pressure with Special Reference to Secondary Rise and Toxicity.

GEORGE MILLES AND PAUL HURWITZ. (Introduced by W. F. Petersen.)

From the Department of Pathology, University of Illinois College of Medicine.

The effect of hypertonic solution on the spinal fluid pressure is of vital importance from a clinical standpoint, since Weed and McKibben[1] show that the cerebrospinal fluid volume could be reduced by intravenous injection of hypertonic solution. Cushing and Foley[2] and Foley and Putnam[3] applied this observation to clinical cases of increased intracranial pressure and it was immediately recognized of importance in the treating of head injuries. However, Browder[4] among others pointed out from clinical observations: first, the toxicity of NaCl solution, and second, the occasional deaths that occur after a short period of clinical improvement after intravenous administration of hypertonic solution in cases of head injuries with symptoms of increased intracranial pressure.

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[4] Reddish, G. F., "The Newer Knowledge of Bacteriology and Immunology," ed. Jordan and Falk, 1928, Ch. XXII. Chicago.

[1] Weed and McKibben, *Am. J. Physiol.*, 1919, **48**, 512.

[2] Cushing and Foley, PROC. SOC. EXP. BIOL. AND MED., 1920, **17**, 217.

[3] Foley and Putnam, *Am. J. Physiol.*, 1920, **53**, 464.

[4] Browder, Jefferson, *Am. J. Surg.*, 1930, —? 1213.

Our experiments were carried out on a series of 10 dogs and one man. We used various concentrations of NaCl and of glucose. The results were uniform. First, it was demonstrated that hypertonic saline has a definitely toxic effect, which in concentrations of 10% is manifested after immediate injection by a primary drop in blood pressure, associated with a rise in cerebrospinal fluid pressure; and in concentrations of 30% uniformly resulted in the death of the animal in doses of 75 cc.

Glucose under no circumstances show toxic effect. From the standpoint of spinal fluid pressure, the primary drop was demonstrated varying in extent, depending on the concentration and volume of the injected material, and persisting for periods varying with same factors. With 10% saline the total period of depression of the spinal fluid pressure was about 50 minutes. With 50% glucose the total period of depression was as long as 4 hours. In every instance, however, a secondary rise followed the primary drop which reached the levels from 20 to 100 mm. of water above the control level. On the basis of this secondary rise which was seen in every instance, we feel that hypertonic solutions of either salt or glucose carry a very great hazard in the presence of intracranial pressure.

5678

Excretion of Organic Phosphorus in Urine.

OLIVE SCHREGARDUS AND HAROLD A. BULGER.

From the Department of Internal Medicine, Washington University School of Medicine, and Barnes Hospital.

Surprisingly large quantities of organic phosphorus in the urine have been recorded in certain pathological conditions. Amounts approaching 1 gm. in 24 hours have been reported and numerous determinations varying from 0.1 to 0.3 gm. may be found. The excretion of so much organically combined phosphorus would constitute a striking abnormality and would bare careful investigation in the light of recent advances in our knowledge of the rôle played by phosphorus compounds in nerve, muscle, and bone physiology. Several investigators did not note these great quantities of urinary organic phosphorus. However, their studies did not include many pathological conditions. A more extended search was, therefore, made to find instances of such an abnormal state.

The organic phosphorus was separated from the inorganic by treating the urine with Mathison's[1] magnesium citrate mixture. The filtrate was tested for complete precipitation by Scott's[2] reagent and an aliquot digested with sulphuric and nitric acids. The digest was neutralized and prepared for colorimetric readings according to Benedict and Theis. It was unnecessary for the subjects of study to be placed on a specific dietary regimen since it has been found that ingestion of various kinds of organic phosphorus compounds does not increase the organic phosphorus excretion.

The following diseases were included in this study: hyperparathyroidism, hypoparathyroidism, hyperthyroidism, diabetes insipidus, diabetes mellitus, chronic arthritis, Paget's disease, amyotrophic lateral sclerosis, syphilis of central nervous system, tuberculous meningitis, epidemic encephalitis, cardiac decompensation, lobar pneumonia, pulmonary emphysema, psoriasis, dermatitis herpetiformis, obstructive jaundice, carcinomatosis, and Hodgkin's disease. The effects of general anaesthesia and the febrile reactions following intravenous typhoid vaccine were also included. The amount of organic phosphorus excreted in the urine was invariably quite small. The average was 13.4 mg. in 24 hours; the maximum 49 mg.

5679

Increase in Cholesterol Content of Gallbladder Bile Following Ligature of Cystic Duct.

ROBERT ELMAN AND J. B. TAUSSIG. (Introduced by Evarts A. Graham.)

From the Department of Surgery, Washington University Medical School.

The action of the gallbladder wall on the cholesterol content of the bile within its lumen has long been the subject of much dispute. To study this problem experimentally a number of different procedures were carried out. One of these consisted in ligating the cystic duct, aspirating a sample of the gallbladder contents and comparing its composition with that obtained several days afterwards. As such a puncture tends to leak it is necessary to tie a ligature around it, which usually causes a more or less severe inflammatory reaction. To obviate the effects of the ligature on the wall of the organ, at the suggestion of Dr. W. H. Cole, gallbladder contents were obtained by puncturing the posterior wall of the viscus, introducing the needle through the liver parenchyma, thus doing away with the necessity of tying a ligature. In some experiments we incised the cystic duct, aspirated through this opening and ligated on either side. In other experiments only a few cc. were removed and the correction due to change in volume by concentration (through absorption of water) or dilution (through the secretion of mucus) taken into account by measuring the bilirubin of the bile each time. In only one case out of seven was there evidence of absorption of cholesterol by the gallbladder. In this experiment there was an increase in the cholesterol concentration of 93% during 2 days but since the bilirubin concentration increased 107% it is obvious that a small amount of cholesterol had disappeared and presumably had been absorbed. This difference is slight and may even be within the range of experimental error. In the other 6 experiments there was a much more marked increase in cholesterol concentration than in the bilirubin. In 3 of them this increase was found in spite of a decrease in the concentration of bilirubin, this decrease being presumably due to dilution from the secretion of mucus. In dog 166 at autopsy nearly all the bile had been absorbed, including the bile pigments, yet there was an abundance of cholesterol crystals found in the shrunken lumen of the gallbladder.

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[1] Mathison, G. C., *Biochem. J.*, 1909, **4**, 233.

[2] Scott, F. H., *J. Physiol.*, 1906-97, **25**, 119.

In 3 other dogs the entire content of the gallbladder was removed and a measured amount of bile reintroduced, keeping a sufficient sample for analysis. This obviated the necessity of bilirubin determinations since the cholesterol content and volume of the bile reintroduced was known. In each case a definite increase, *i. e.,* 18, 49, and 300% in the total amount of cholesterol in the bile during its sojourn (2, 3 and 16 days respectively) in the gallbladder. Since the cystic duct was closed we may infer that the increase came from the wall of the gallbladder.

The rôle of infection was noted incidentally in many of these experiments by study of the gallbladder wall at autopsy. Unfortunately these observations were limited. Most of the specimens showed no evidence of inflammatory change. In dog WH in which definite changes were noted, however, the increase in cholesterol was quite marked. On the other hand, in Dog B45 in which the greatest increase (300%) in the cholesterol content of the gallbladder bile was found, no inflammatory changes were made out. However, this experiment was of 16 days' duration, and an acute inflammation may have occurred and subsided, leaving no histological trace.

The high cholesterol content of the peritoneal fluid in Dog 830, where there was a localized peritonitis, may be explained by the fact that inflammatory exudates in general may contain much cholesterol.[1] The method for quantitating cholesterol was the colorimetric one and will be described in detail elsewhere. That used for bilirubin is described in a paper by Rous and McMaster.[2]

5680

Addition of Cholesterol to Hepatic Bile Subjected to Gallbladder Influence.

ROBERT ELMAN AND J. B. TAUSSIG. (Introduced by E. A. Graham.)

From the Department of Surgery, Washington University Medical School.

To study the influence, if any, of the gallbladder on the cholesterol content of bile, a series of experiments were performed in which a "partitioning ligature" was so placed[1] that 2 portions of the liver were drained into separate rubber balloons through intubation of the common duct. One part of the bile flowed directly from the liver, whereas the other part was subjected to gallbladder influence, before flowing into the collecting bag. This device was first used by Rous and McMaster[1] in their classic studies on the concentrating activities of the gallbladder.

Dog. I, weight 12 kg., under ether anesthesia a ligature was placed around the common duct just proximal to 2 hepatic ducts which were then made to drain directly into a rubber balloon intubated at the termination of the choledochus. A second balloon was connected with the common duct proximal to the ligature. This drained the rest of the liver, including the gallbladder which, however, was first completely emptied. The abdomen was closed in layers and the bags left therein. Five days later the dog, in excellent condition, was killed with chloroform. The upper bag (in connection with the gallbladder) contained 105 cc. of cloudy infected bile and was found to have drained 90 gm. of liver. The lower bag (from the liver directly) contained 205 cc. of clear bile and had drained 225 gm. of liver. Bilirubin comparisons to evaluate the degree of concentration by the gallbladder could not be accurately made because of the presence of infection in one of the specimens. Comparison of the cholesterol content revealed striking differences. The gallbladder bile contained 1.1 mg./cc., the hepatic bile but 0.06 mg./cc., values in accord with many estimations of isolated gallbladder and hepatic biles from many dogs, as will be reported later. The difference in the bile subjected to gallbladder influence is even more evident if one calculates the output per gm. of liver drained. The bile in the upper (gallbladder) bag contained 1.3 mg. of cholesterol per gm. of liver drained, the lower one but 0.05 mg. That concentration by the gallbladder could not account for these differences is obvious from the fact that 105 cc. and 225 cc. were put out by the 2 portions of the liver.

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[1] Wells, H. G., Chemical Pathology, 5th Edition, Philadelphia, 1925, 301.

[2] Rous, P., and McMaster, P. E., *J. Exp. Med.*, 1921, **34**, 47.

[1] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1921, **34**, 47.

In 5 other dogs a simpler method of performing this experiment was adopted by using only one bag, emptying the gallbladder either by puncture through the wall (introducing the needle behind through the liver parenchyma to avoid the necessity of tying the puncture hole) or by passing a catheter into it through the opening in the common duct.

The output of bile pigment per gm. of liver drained was (with the exception of dog BB) almost exactly the same from each part of the liver. This agreement was taken as evidence that the bile flowing into the gallbladder and into the balloon from the 2 parts of the liver was primarily the same. Subsequent differences therefore could only be due to the action of the gallbladder on the bile flowing above the partitioning ligature. Since the experiment lasted but 48 hours the pressure in the isolated gallbladder system could not have become great enough to influence secretion.

The results confirm, as to the bile pigment content, the concentrating action of the gallbladder. The cholesterol of the gallbladder contents, however, was in each case greater than mere concentration of the bile could account for. This can be best seen by calculating the cholesterol output per gm. of liver drained. In the 5 dogs studied the gallbladder bile contained 153%, 122%, 27%, 18% and 67% *more* cholesterol per gm. of liver drained than the hepatic bile from the rest of the liver. These results indicate that in some way cholesterol was added to the bile by the gallbladder wall.

The rôle of infection seems to be an important one, first as shown by the first experiment described (Dog I) in which the infected gallbladder bile contained a very high content of cholesterol. In Dog Y, in which acute inflammatory changes were noted in the section of gallbladder wall, the greatest amount of the cholesterol was found in the gallbladder bile. Sufficient study of this factor, however, has not been made, although there is other evidence to be mentioned later which suggests also that infection may increase the output of cholesterol by the walls of the biliary tract.

Some of these dogs were fed large amounts of fat-containing food, but the effect of a high cholesterol diet is difficult to judge from these few experiments. There is, in general, much conflict of opinion as well as of evidence on this point.

5681

Cholesterol Content of "White Bile" from Various Sources, Including Contents of "Hydrops" of Gallbladder.

ROBERT ELMAN AND J. B. TAUSSIG. (Introduced by E. A. Graham.)

From the Department of Surgery, Washington University Medical School.

During experiments on the biliary tract of dogs a number of instances of so-called white bile were observed, one from the gallbladder and the others from the ducts. Several human cases were also encountered in which hydrops of the gallbladder was found at operation. These specimens were all analyzed for their cholesterol content.

The significance of these observations concerns the question of the function of the gallbladder and bile ducts as regards the cholesterol of the bile. Is cholesterol absorbed or is it secreted by the bile duct and gallbladder epithelium? Or is it indifferent to the activities of these cells? Study of "white bile" furnishes one way of getting suggestive information as to these questions since it is well known that this secretion is a product not of the liver but of the duct mucosa. The colorless fluid found in hydrops of the gallbladder must similarly be a product of its wall since in these cases the cystic duct is occluded.

In 4 cases of hydrops in the human 2 gave 0.19, 0.45 mg. per cc. of cholesterol, and 2 cases gave macroscopic cholesterol crystals. Stepp and Nathan[1] found 0.64 mg./cc. of cholesterol in the contents of an "acute hydrops" of the gallbladder. Winternitz,[2] however, in another case finds "cholesterol absent". The method he used is not mentioned. Naunyn[3] states that cholesterol is present in such fluid, but always in small amounts. Fowweather and Collinson[4] found up to 0.92 mg./cc. in 9 cases. In one of our cases (G. C. C.) no evidence of inflammation was made out on section of the wall, but since the lesion had been present for some months with no acute symptoms it is probable that it had subsided, leaving no trace. The other 3 cases all showed evidence of inflammation.

The findings in dogs include 5 instances in which the colorless secretion of the common duct, isolated by ligature, was collected and analyzed. They all contained cholesterol in concentrations varying between 0.047 and 0.085 mg./cc. values somewhat less than that present in normal (hepatic) bile in dogs as found in many isolated determinations by us. Rous and McMaster[5] report that "cholesterol is practically absent as shown by the Lieberman-Burchard test which is occasionally negative and at most weakly positive" in similar specimens of "white bile." The only one of our specimens which was infected contained much more cholesterol, 0.2 mg./cc., or over twice that of the highest value of the other 4 specimens. That infection tends to increase the output of cholesterol is also shown by the high values in the other 2 specimens, one from the infected dilated hepatic ducts following a total biliary obstruction and the other from an infected gallbladder fistula (cystic duct tied). These contained 0.22 and 0.50 mg./cc. of cholesterol respectively. That the finding of cholesterol is not due to its general presence in all body fluids is shown by control analyses of various samples of saliva, and ganglion cyst fluid, in which measurable amounts could not be detected.

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[1] Stepp, W., and Nathan, M., *Med. Klin.*, 1919, **15**, 40.

[2] Winternitz, H., *Z. f. Physiol. Chem.*, 1896, **21**, 386.

[3] Naunyn, B., *Klinik der Cholelithiasis*, Jena, 1892, 106.

[4] Fowweather, F. S., and Collinson, G. A., *Brit. J. Surg.*, 1926, **14**, 583.

[5] Rous, P., and McMaster, P. E., *J. E. I.*, 1921, **34**, 75.

5682

Allergic Reactions in Polio-Convalescent Children After Intracutaneous Injection of Heat-Inactivated Poliomyelitis Virus.*

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The experiments here reported represent a logical continuation of work previously published,[1] on the accelerated febrile response of convalescent monkeys to reinoculation with poliomyelitis virus. We interpreted our findings as indicating that recovery from poliomyelitic infection left the animals in a state of hypersensitiveness against the specific agent of the disease. The present paper purports to show that a similar condition may be demonstrated in children who have convalesced from a previous attack of infantile paralysis. We are much indebted to Dr. Charlton Wallace, Surgeon-in-Chief, and to Drs. J. M. Moore and F. J. O'Malley, Senior and Junior Resident Physicians of the New York State Reconstruction Home at West Haverstraw, N. Y., for their most helpful support in this investigation.

Twenty-seven children and youths (3 to 22 years old) with a proven history of a previous attack of poliomyelitis several years ago, were inoculated intracutaneously on one arm with 0.2 cc. of the Berkefeld filtrate of a 5% monkey virus emulsion, which had been inactivated by heating for one hour at 65°C.† A similar amount of a filtered and heated 5% emulsion of normal monkey cord was injected intracutaneously on the other arm. Both preparations had been tested before use by culture and animal experiment not only for bacteriological sterility (including *B. tuberculosis*) but more particularly for freedom from live virus by intracerebral inoculation of one monkey. All of the 27 individuals reacted to the killed virus in a typical allergic manner with local redness and slight swelling, which increased steadily from the fifth hour to the 24th hour after inoculation. A slight urticarial reaction which appeared in some children during the first 2 hours after injection of the control material completely subsided over night, so that only the prick from the needle wound could be detected. The specific reactions were easily read after the 24-hour interval. The area of erythema was usually about 3 by 4 cm. in diameter, few reactions exceeding and fewer yet not attaining these dimensions. In almost half of the cases there was a darker center surrounded by a lighter halo. The reactions faded out rather rapidly, so that with occasional exceptions only a local area of browning persisted at the end of the 48-hour interval. Four more convalescent children were inoculated subcutaneously, 2 receiving 0.5 cc. of the killed virus filtrate and 2 a similar dose of the normal cord filtrate. The 2 individuals injected with the control material exhibited no local reaction whatsoever, while the 2 which had received the virus material showed marked redness and induration around the point of inoculation. In no case was there any significant change of the body temperature.

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* Under a grant from the International Committee for the Study of Infantile Paralysis, whose work is being financed by Jeremiah Milbank.

[1] Jungeblut, C. W., *J. Exp. Med.*, 1931, **53**, 159.

† We have since found that different filtrates may vary considerably in potency and that prolonged extraction of the cord is essential in order to obtain a maximum concentration of reacting substances in the filtrate.

Our experience with normal individuals, although too limited at present to warrant detailed discussion, indicates a preponderance of positive reactions in adults over those obtained in younger children. A correct interpretation has frequently been made difficult by the tendency of adults to react with the control material.

Although we had failed before to obtain clearcut evidence for the possibility of producing local reactions in convalescent monkeys under similar conditions, we believe there can be little doubt that the febrile reaction in the monkey and the local reaction in the human are manifestations of the same state of allergy against the virus. If it is true, as the trend of present research would suggest, that the majority of older children and adults are protected against poliomyelitis by past latent infection, which in turn has induced virucidal antibodies, we should expect to obtain a large percentage of positive reactions in normal individuals of the older age groups and a smaller incidence among children under 5 years of age. A test in that form might then conceivably serve as a means of recognizing susceptibility to the disease. Experiments are now under way to compare the skin reactivity with the content of neutralizing substances in the serum.

5683

Lipid Metabolism of Tumors. II. Influence of Food Lipids upon the Nature of Tumor Phospholipid.

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That the nature of the lipids in the animal body is influenced by the food lipids has been reported by previous workers. The question has been raised in connection with the lipid metabolism of tumors whether or not the same can be said about the tumor lipids or to what extent they are influenced by the lipids of the food.

The tumors used in this experiment are the Jensen rat sarcoma, mouse carcinoma of Buffalo Strain 3 and the Crocker sarcoma of the mouse. The animals after the inoculation of the tumor were fed food composed of casein (30.6%), cane sugar (40.8), lipid (21.7), and salt mixture (4.9), with yeast and Oscodal as the sources of the vitamines. As the lipids of the food cod liver oil,

TABLE I.
Phospholipid Iodine Number of Liver and Tumor of Rats Fed With Different lipids.

Main lipid in the diet	Tissues		Dry substance %	Phospholipid		Weight	
				gm. per 100 gm. dry tissue	Iodine number	Body gm.	Tumor gm.
Cod liver oil	Tumor	1	17.62	7.57	104	175	20
	"	2	17.05	8.16	93	242	31
	"	3	17.45	7.34	97	174	36
	Liver	1	29.00	11.18	130		
	"	2	27.65	10.80	127		
	"	3	27.50	9.40	133		
Butter	Tumor	1	21.30	6.62	88	164	50
	"	2	17.82	6.30	75	247	59
	"	3	16.55	7.14	80	201	78
	Liver	1	33.80	6.67	97		
	"	2	25.30	8.95	85		
	"	3	22.60	10.30	84		
Linseed oil	Tumor	1	17.55	6.70	97	194	35
	"	2	22.10	5.30	100	281	84
	"	3	17.35	5.20	96	164	50
	Liver	1	27.70	10.08	108		
	"	2	33.20	7.50	114		
	"	3	25.80	10.93	111		
Cocoanut oil	Tumor	1	17.85	8.50	85	205	26
	"	2	17.80	8.94	75	320	26
	"	3	16.95	8.99	85	217	41
	Liver	1	26.10	11.09	94		
	"	2	27.30	11.23	102		
	"	3	26.85	8.40	100		

butter, linseed oil and cocoanut oil, that is, the high and low iodine number animal and vegetable lipids, were used. After a considerably long time of feeding with these special diets, 5 to 7 weeks in case of the rat tumor and about 2 months in the other cases, the animals were killed and the iodine number of the tissue lipids was determined by the micromethod in which pyridine sulphate dibromide of Rosenmund and Kuhnhenn was used as a halogenizing agent and the lipids by the oxidative method of Bloor. The accuracy of this method for iodine number compared with macromethod was discussed, and the conclusion that this micromethod is available for the iodine number determination was emphasized. The phospholipid iodine number of the tissue lipids of the tumor animals was first studied. The results are shown in the following tables, and in summary it may be said that the iodine number of the phospholipid of tumors is dependent upon the food lipids like that of other tissues of the body. The iodine number of tumor phospholipid is lower than that of liver. The brain phospholipid of the mouse does not seem to change its iodine number by 2 months' feeding with different lipids.

TABLE II.

Phospholipid Iodine Number of Tumors and Other Tissues of Mice Fed With Different Lipids.

Tumors: Adenocarcinoma, Buffalo Strain 3; Sarcoma, Crocker.

Tissues of 6-10 mice were combined, from which adequate amounts of various tissues were taken for the extraction of the lipids.

Main lipid in the diet	Tissues	Dry substance %	Phospholipid	
			gm. per 100 gm. moist tissue	Iodine number
Cod liver oil	Carcinoma	18.55	1.000	100
	Liver	29.20	3.235	122
	Kidney	21.03	1.985	116
	Spleen	22.10	1.585	102
	Heart		2.280	142
	Brain		6.425	81
Butter	Carcinoma	20.00	1.735	97
	Liver	28.10	3.005	112
	Kidney	23.00	2.300	115
	Spleen	23.05	1.320	104
	Heart		2.100	138
Cocoanut oil	Sarcoma	19.20	0.905	63
	Liver	27.40	2.430	90
	Kidney		2.490	98
	Spleen		1.665	88
	Heart		2.520	107
	Brain		5.825	87

5684

Effect of Copper and Iron on Hemoglobin of the Rat in Nutritional and Hemorrhagic Anemias.

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Following Hart, Steenbock[1] et al. on the influence of minute amounts of copper in improving the hemoglobin in rats with nutritional anemia it seemed desirable to determine whether or not this same thing could be obtained in the anemia of the rat induced by repeated bleedings.

We have compared two series of rats, (1) a group made anemic by long continued milk diet; (2) a group of normal animals made anemic by repeated bleedings. In both groups the animals were of the same age and weight, and treated exactly alike in every other particular.

Group I. Nutritional Anemia Group. These rats were kept on a diet of milk until they showed a definite anemia, and then divided into 3 sub-groups. The first sub-group was kept on milk only. The second sub-group was kept on milk with the addition of iron and copper. The third sub-group was kept on milk with iron only. The amount of iron used was 3 mg. per rat per day given in the form of ferric ammonium citrate. The amount of copper used was one-tenth milligram per rat per day, in the form of copper sulphate. We had on hand a considerable amount of the iron salt from our former experiments, and this was carefully analysed for the presence of copper as an impurity, and was found to contain only very slight traces of the latter metal.

After a preliminary examination of the blood of a few trial animals had shown that they had become sufficiently anemic the hemoglobin of all the animals was determined spectrophotometrically. Where relative results are wanted this is the most accurate method at our disposal for the determination of hemoglobin. These figures are recorded in each sub-group under the caption "First determination." On the twenty-fourth and fifty-fourth days afterwards other determinations are made, which are recorded in the corresponding columns of each sub-group.

Table I summarizes results for 54 days; in those fed on milk only

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[1] Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

SUMMARY OF TABLE I.
Nutritional Anemia.

	1st Determination	2nd Determination	3rd Determination	No. of Rats
Diet of milk only	0.829 Var $\frac{0.915}{0.729}$	0.785 Var $\frac{0.871}{0.696}$	0.662 Var $\frac{0.810}{0.535}$	26
Milk plus copper plus iron	0.785 Var $\frac{0.874}{0.667}$	0.984 Var $\frac{1.017}{0.918}$	0.937 Var $\frac{1.008}{0.842}$	23
Milk plus iron	0.821 Var $\frac{0.908}{0.715}$	0.874 Var $\frac{0.924}{0.735}$	0.692 Var $\frac{0.785}{0.589}$	24

Note: The figures given are averages. Such determinations as were greater than the average were again averaged, and this result is the numerator of the fraction. In the same way, the denominator is the result of treating those determinations below the average in the same way.

the hemoglobin was lowered from an average of 0.829 to 0.662. In the sub-group on milk with iron only added the hemoglobin went down to approximately the same extent, from 0.821 to 0.692.

It is interesting to note in this group in the determination made 24 days after that there is a slight rise, namely to 0.874, hardly more than a normal variation, and this moreover is not maintained.

In the sub-group on milk with the addition of both iron and copper there is a very striking improvement. Beginning with a concentration of 0.785 there is an immediate increase in the hemoglobin to 0.984 which remains up to 0.937 on the fifty-fourth day.

To any one thoroughly familiar with the spectrophotometer the difference is much more striking than these figures would indicate, and it was almost possible to pick out the rats which had copper at the first glance through the instrument.

This confirms the work of Hart and Steenbock that in the nutritional anemia of the rat the addition of a minute amount of copper to the diet produces a prompt and satisfactory increase in the hemoglobin, whereas the addition of iron alone not only fails to produce an increase in the hemoglobin but fails even to check the progressive development of anemia.

Hemorrhagic Anemia. The second set of experiments concerned itself with the anemia produced by abstraction of blood. A hemoglobin determination was made on a large group of healthy animals. They were then placed on the standard iron-free (iron-poor) diet used in our previous work and then divided into 2 similar groups and the following day all were bled one-third of their blood volume. A similar amount was removed again 7 days later. Subsequent hemoglobin determinations were made on the twenty-fourth and seventy-first days after bleeding. The results are summarized in Table II.

SUMMARY OF TABLE II.
Hemorrhagic Anemia.

	Before Bleeding	24 Days After Bleeding		No. of Rats
Control Group (Practically iron-free)	0.821 Var $\frac{0.899}{0.731}$	0.708 Var $\frac{0.787}{0.627}$	0.817 Var $\frac{0.886}{0.723}$	49
Copper and iron group	0.803 Var $\frac{0.898}{0.725}$	0.716 Var $\frac{0.809}{0.633}$	0.791 Var $\frac{0.914}{0.641}$	51

The results are very different from those obtained in the nutritional group. The rats in the sub-group receiving copper and iron in addition to the diet had a concentration of 0.803 before and 0.791 on the seventy-first day after bleeding. An intermediate reading was taken to determine the rate of change. In other words, in this group the hemoglobin was almost exactly the same on the seventy-first day as in the beginning, whereas in the nutritional group, a marked improvement occurred as early as the twenty-fourth day, which remained at substantially the same level on the fifty-fourth day. In the sub-group used as control, we find a concentration of 0.821 before and 0.817 on the seventy-first day after bleeding. Expressed in another way, the group treated with the addition of iron and copper to a standard diet did not improve either more rapidly or to a greater degree than those on a standard control diet alone.

Conclusions: In rats with nutritional anemia the addition of iron and copper to the diet produces definite and prompt improvement in the hemoglobin concentration. In rats with anemia produced by repeated abstraction of blood the addition of iron and copper to the diet produces no perceptible effect.

5685

Fermentation of Glucosamine Hydrochloride by Bacteria.

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With the exception of a few observations on glucosamine the fermentation reactions of micro-organisms on the hexosamines have not been studied. Inasmuch as these nitrogenous sugars contain a carbohydrate and an amino group, both of which are necessary for the growth of bacteria, an investigation of the metabolic activities of different organisms on these substances might be of value. Abderhalden and Fodor[1] reported that *B. tenuis* fermented glucosamine hydrochloride. Meyer[2] found that glucosamine hydrochloride was fermented by such organisms as he used in the same manner as glucose is fermented with the exception of *B. paratyphosus* A, which formed acid only in glucosamine. Noble and Knacke[3] found variations in the ability of different strains of *B. diphtheriae* and diphtheroid organisms to ferment glucosamine hydrochloride but no relationship between these differences and virulence. The organisms capable of fermenting glucose all fermented glucosamine hydrochloride except *B. mesentericus,* yeast and possibly *B. proteus.* The amount of acid formed by *B. proteus* was slight, the reaction delayed and transient, and therefore doubtful whether or not due to the fermentation of the glucosamine. *B. paratyphosus* A formed gas in glucosamine hydrochloride. This is contrary to the findings reported by Meyer.

B. coli and *B. mucosus capsulatus* grew well in a medium containing only inorganic salts and glucosamine hydrochloride. There was an accumulation of free ammonia in the medium indicating that deaminization is one part of the mechanism by which bacteria split a molecule of glucosamine. It is probable that it is the initial step leaving the hexose free to be acted on as such because in all cases observed, when an organism ferments glucosamine, the fermentation proceeds in the same manner as when that organism is acting on any monosaccharide.

There is still a doubt whether the sugar in the glucosamine molecule is glucose or mannose. With this in mind parallel tests were made with the same organisms using glucose, mannose and glucosamine hydrochloride.

The results are somewhat confusing. *B. mesentericus* ferments glucose but not mannose or glucosamine hydrochloride, *B. anthracis* ferments glucose and glucosamine hydrochloride but not mannose, and yeast ferments glucose and mannose but not glucosamine hydrochloride. Thus we have organisms that can ferment glucose unable to ferment glucosamine hydrochloride and also organisms able to ferment mannose that can not use glucosamine hydrochloride. It would therefore appear that the kind of sugar in the glucosamine molecule is not the only factor that determines whether an organism can or can not utilize this substance. The presence of the amino group and especially the carbon atom to which it is attached may be the inhibiting factor. In glucosamine the amino group is attached to the second carbon atom. There are other hexosamines in which the amino group is attached to the first, the third and the sixth carbon atom. These hexosamines are being prepared and the action of bacteria on them will be investigated.

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[1] Abderhalden and Fodor, *Zeit. f. Physiol.,* 1913, **87**, 214.

[2] Meyer, *Biochem. Zeit.,* 1913, **57**, 297; 1914, **58**, 415.

[3] Noble and Knocke, *J. Bact.,* 1928, **15**, 53.

5686

Effect of Diathermy on Concentration of Complement and Normal Opsonins.

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From the Department of Bacteriology, Northwestern University Medical School.

The increasing use of diathermy has augmented the need for greater knowledge of its effect upon the body. Such knowledge may aid in the elucidation of some controversial points in immunology since a temperature is produced without the injection of protein or other foreign substance. Nine cases of allergic asthma were treated with diathermy according to the method described by Neymann.[1] The investigation was limited to a study of the concentration of complement and natural opsonins, and the frequency and choice of time of procuring samples were restricted. The number of treatments was 1 to 4, the time of second treatment, 2 hours to 17 days, and the change in opsonic index in 10 cases was 0.1 to 0.4. Serum from a normal adult, the same throughout the experiment, was used as a control.

Complement concentration of the patient's serum was determined in the usual way employing sheep's red blood cells and rabbit anti-sheep cell serum with appropriate controls. The results of such tests demonstrated that no significant change in the complement content of the serum was effected by diathermy and such slight alterations as did occur are probably explainable by normal variation in complement or slight differences in the suspension of blood cells.

Opsonins. A modification of the opsonic test used by Tunnicliff[2] was selected as most applicable for the conditions and material at hand. A heat killed laboratory strain was used, leukocytes from a normal control and unheated serum from patients and control. The opsonic index was obtained by dividing the percentage phagocytosis occurring with patient's serum by that with the control serum. It is probable that the minimum significant difference by this method is 0.5. Therefore, with the possible exception of case III, the changes are not noteworthy.

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[1] Neymann, *J. Am. Med. Assn.*, 1931, **96**, 7.

[2] Tunnicliff, *J. Am. Med. Assn.*, 1926, **87**, 625.

Summary. Diathermy did not produce a significant change in complement concentration nor in opsonic index within the limits of this investigation. It is apparent that a complete report can come only from a large number of cases with frequent tests at selected times.

5687

Cholesterol and Bile Pigment Changes Incident to Diets Low in Protein.

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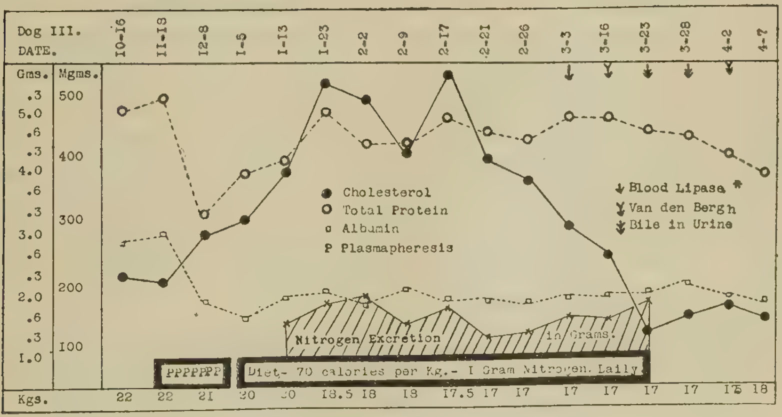
Variable elevations of blood cholesterol have been observed during the past 5 years both clinically and experimentally where nitrogen balance was disturbed. In order to determine the possible importance of nitrogen balance on cholesterol levels, 6 dogs were placed on diets adequate in regard to minerals, vitamines and calories, but deficient in nitrogen. The nitrogen was limited to 1 gm. per day per animal. On this diet the urinary nitrogen excretion



* Olive oil lipase.

CHART 1.

ranged from .99 to 2.2 gm. per day; so that a negative nitrogen balance was established and could be maintained for any desired period.

All dogs placed on this diet showed striking elevations in their total blood cholesterol and a gradual loss of weight. After 2 to 5 weeks on the low nitrogen diet, blood cholesterol began to increase gradually. It increased from the control readings of 125-225 mgm. to 450-550 mgm. after 4 to 12 weeks. If the diet was discontinued, a very slow return of cholesterol to the normal level was observed after many weeks. If the low protein diet was continued, the wasting became marked and a gradual drop in blood cholesterol began. After the cholesterol had dropped near to or below normal, blood lipase[1] (olive oil lipase) ordinarily appeared in increasing amounts. Later the Van den Bergh reaction became positive, soon bile appeared in the urine and the animal would refuse to eat. Cautious feeding of milk followed by a gradual return to the general ration would restore the animal only after several months. Realimentation with liberal amounts of meat was associated with a peculiar toxic state and resulted in the death of one animal not unlike that seen with meat feeding in cirrhosis of the liver in dogs. Total serum proteins and serum albumin fell 1½ to 2% but failed to reach the critical levels for edema formation. A reduction of the total serum protein by plasmaphaeresis showed a return to near normal levels even though a severe negative nitrogen balance was maintained. (See chart.) Edema was noted in one animal on realimentation and this edema persisted for 6 weeks.

Many details of this work are being followed and will be reported later but cholesterol has been regularly elevated from 200 to 400 mg. in the dogs on a diet adequate except for nitrogen. Prolonged diets deficient in nitrogen seemed to produce liver damage first indicated by a fall in blood cholesterol. / This would support recent observations on cholesterol in parenchymatous liver damage in man.[2] Marked wasting of the body fat and muscle occurred and total serum proteins were slightly reduced, but edema did not appear except in one instance and that occurred on realimentation. There was no relation between cholesterol and total protein levels or the state of water balance.

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[1] Crandall, L. A., and Cherry, Ian S., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 572.

[2] Epstein, Emanuel Z., *Arch. Int. Med.*, 1931, **47**, 82.

5688

Possible Etiologic Factors in the Production of Pulmonary Osteoarthropathy.*

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(Introduced by Edmund Andrews.)

From the Department of Surgery, University of Chicago.

Periosteal new bone formation secondary to intrathoracic lesions such as pneumonia, empyema, bronchogenic carcinoma or tuberculosis, spoken of as pulmonary osteoarthropathy or generalized osteophytosis,[1] is a well recognized clinical entity. Theories of the factors responsible for the new bone formation have varied from that of toxic absorption from focal infections in the chest[2] to changes in the acid-base equilibrium through decreased aeration as a result of constriction of the bronchi or of lung compression and collapse.

To explain the etiologic factor responsible for these peripheral bone changes, we have attempted to produce them in experimental animals. Before and after creating intrathoracic complications in dogs, roentgenograms were made of the long bones of the lower extremities and the calcium and phosphorus and carbon dioxide content and H ion concentration of the blood serum were determined. These studies were repeated at varying intervals and no significant changes in the blood chemistry were found.

Of 9 dogs, into whose right pleural cavity from 100 to 300 cc. of parafin were injected, one survived 9 months and 2 are still alive after a period of nearly 18 months. In each instance, in addition to the mechanical pressure on the lung by the parafin, there was a prompt pleural effusion which almost completely displaced air-containing tissue on the right half of the chest. No periosteal reaction or new bone formation along the shafts of the long bones could be demonstrated in any of these dogs although frequent x-ray examinations were made. Blood chemistry studies were made at frequent intervals. No consistent or significant blood changes were noted following the injection of the parafin.

Using a method described by one of us,[3] collapse of one or more

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research.

[1] Crump, *Virchows Archiv. f. Path. Anat.*, 1929, **272**, 467.

[2] Mills and Mumey, *Arch. Int. Med.*, 1929, **43**, 516.

[3] Adams, W. E., and Livingston, H. M., *Ann. Surg.*, in press.

lobes of the lungs of 13 dogs was accomplished. One of the dogs developed a lung abscess and lobectomy was performed upon 5 dogs at varying intervals following collapse of the lobe or of the corresponding lung. Roentgenograms were taken and examinations were made of the calcium and phosphorus and carbon dioxide content and of the H ion concentration of the blood serum of these dogs. In no instance were we able to demonstrate changes in the long bones similar to those described as pulmonary osteoarthropathy and there was no consistent change in the blood chemistry.

We were thus unsuccessful in our attempts to produce new periosteal bone by pressure upon a lung from a foreign body in the pleural cavity; stenosis of a primary or secondary bronchus; collapse or total absence of a lobe or of an entire lung; pleurisy with effusion; and solitary lung abscess. It should be pointed out, however, that none of the above procedures produced any marked respiratory embarrassment and that they were not attended by any definite changes in acid-base balance or in the calcium and phosphorus content of the blood serum. More extensive studies are being undertaken to explain this strange clinical phenomenon.

5689

Agglutinins Produced by the Injection of Related and Unrelated Antigens.

ROY F. FEEMSTER. (Introduced by C. W. Duval.)

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In making observations upon the formation of agglutinins in response to injection with typhoid vaccine it was noted that there was a wide individual variation in the response. Some students who had repeatedly received typhoid vaccine on previous occasions failed to develop agglutinins when injected with typhoid vaccine in this department. Others who had never received typhoid vaccine previously developed high titers. The problem presented itself of determining whether this difference was due to a more responsive antibody-forming mechanism in general or due only to the individual's response to a particular antigen.

Forty-five students were given the routine 3 doses of TAB vaccine, and in addition were given 3 doses of a vaccine containing *Staphylococcus aureus* and a coccus isolated from the blood of a case of measles by Drs. Duval and Hibbard. Macroscopic agglutination tests were performed for all 5 of the above organisms before injection, and again 10 days after the last dose of vaccine.

A glance at the parallel agglutination tests gave the impression that there was absolutely no relation between the ability to produce typhoid agglutinins and staphylococcus agglutinins, the latter being of course entirely unrelated to the typhoid bacillus. The impression was confirmed by the correlation coefficient (–.00031 ± .101). On the other hand there appeared to be a high degree of relation between the ability to produce typhoid and *B. paratyphosus A* agglutinins, which are related antigens. This was likewise confirmed by the correlation coefficient (+.302 ± .091). Unfortunately the *B. paratyphosus B* and the measles coccus were poor antigens, failing to stimulate a sufficient amount of agglutinins from which to draw conclusions.

These observations would seem to indicate that the response of an individual to an antigen depends not so much upon a sensitive immunity mechanism in general as to the sensitivity of the individual to the particular antigen.

5690

The Antibody Response to Injection with Typhoid Vaccine.

ROY F. FEEMSTER. (Introduced by C. W. Duval.)

From the Department of Pathology and Bacteriology, Tulane University.

Incidental to the investigation of another problem opportunity was afforded to check the results reported in a previous study upon the significance of the antibodies produced by typhoid vaccine.[1, 2, 3] Forty five students were injected with typhoid vaccine. Agglutination reactions were performed before the injection, and again 10 days after the third dose of vaccine. The results were analysed in the same manner as in the previous study.

It was quite upsetting to note that in this case those who had not had previous vaccine responded by a marked agglutinin production (1/300) instead of a low average (1/66) as before, completely reversing one of the conclusions drawn in the former work. On the other hand, those who had had typhoid fever responded in

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[1] Feemster, Roy F., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 636.

[2] Feemster, Roy F., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 911.

[3] Feemster, Roy F., to be published in an early number of the *J. Infect. Dis.*

about the same manner this time (1/288) as before (1/285). Likewise those who had had previous typhoid vaccine produced agglutinins in comparable titers in this series (1/113) as in the previous group (1/162).

It is interesting to note, however, that when the 2 groups are combined, so that we have a total of 135 individuals instead of 45, the averages again return to the relation found in the first experiment, those who had had typhoid fever averaging 1/287, those who had had previous typhoid vaccine 1/143, and those who had had neither 1/115. The reversal of the average in the one group in the second experiment was produced by the high agglutinin titers of 4 students. There were individuals with just as high titers in the previous study, but chance happened to throw 4 such students into a small group of 9 in the latter instance and there were only 4 with titers as high in a group of 34 in the first experiment. No doubt if there were any way of proving it, we would find that these individuals belong in the group of so-called natural immunes occurring in the population in general.

Practically all of the other conclusions drawn in the study made previously are confirmed by this second series of observations, and the figures will not be repeated here.

This reversal of averages in a small series emphasizes the danger of drawing conclusions from too few observations. If the second experiment had been the only one performed the author would no doubt have published a note including a conclusion which has been drawn by others from similar small series of observations, but which appears to be the reverse of the actual facts.

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Some Effects of the Vitamin B Complex on Appetite and on Utilization of Food.

CLAIRE E. GRAHAM AND WENDELL H. GRIFFITH.

From the Laboratory of Biological Chemistry, St. Louis University School of Medicine.

The relation of the vitamin B complex to the rate of growth, to the consumption of food and water and to the utilization of food in rats has been studied. The Evans and Burr diet (purified casein—25, sucrose—75, salt mixture—4) was used and was sup-

TABLE I.

Group	No. of rats	No. of days	Total gain in wt.		Total calories	Daily Dietary Supplement*
			low-high	average		
Restricted			gm.	gm.		
1	11	40	93-113	102	1365	0.5 gm. yeast
2	12	40	95-111	103	1365	0.5 gm. yeast + 0.5 gm. liver
3	12	40	92-118	104	1365	0.15 gm. yeast + 0.85 gm. liver
4	10	40	90-125	108	1380†	5 drops tikitiki + 0.1 gm. autoclaved liver
Ad Lib.						
5	10	80	161-238	204	——	Stock control diet
6	14	80	141-191	163	3230	0.5 gm. yeast
7	10	80	155-266	207	3440	0.5 gm. yeast + 1.0 gm. autoclaved liver
8	10	80	109-152	131	2660	1.0 gm. liver
9	10	80	138-204	179	3284	0.15 gm. yeast + 0.85 gm. liver
10	10	80	171-240	202	3710	0.5 gm. yeast + 0.5 gm. liver
11	12	80	142-212	186	3330†	5 drops tikitiki + 0.2 gm. autoclaved liver
12‡	12	80	162-275	219	3570†	5 drops tikitiki + 1.0 gm. autoclaved liver

* Each rat received 9 drops cod liver oil daily.

† Tikitiki not included in calculated value.

‡ Six of these rats received 10 drops of tikitiki daily during the first half of the experimental period. Inasmuch as the extra tikitiki was without effect the daily supplement was decreased to 5 drops during the remainder of the experiment.

plemented daily with 9 drops of cod liver oil. The B complex was supplied by daily additions of one or more of the following: whole dried hog liver (high B_2–low B_1), autoclaved liver (high B_2), whole dried yeast (high B_1–moderately high B_2), tikitiki (high B_1). Male rats were placed on the experimental diet when 50 gm. in weight and were kept in individual raised cages. Some of the data are shown in Table I. Groups 1 to 4 were restricted in food consumption to approximately the same number of calories. The remaining groups were fed the basal diet *ad libitum.*

The weight increments for the first 4 groups were surprisingly uniform in spite of the fact that only one of the 4 supplements (Group 2) was adequate for optimum growth when the basal diet was fed *ad libitum* (Group 10). The results with Group 4 were particularly interesting because of the relatively low concentration of vitamin B_2 required for satisfactory utilization. In these experiments the utilization of food was apparently unaffected by considerable variation in the amounts of B_1 and B_2 furnished in the supplements.

On the other hand, the appetite of the rats was markedly affected by the nature of the supplement. If either tikitiki (B_1) or autoclaved liver (B_2) was used as the source of the B complex appetite failed and growth did not occur. However, the combination of tikitiki and autoclaved liver resulted in optimum growth (Group 12); i. e., growth as good or better than that of the stock control rats (Group 5). The difference between Groups 6 and 7 and between Groups 11 and 12 was largely due to the appetite-stimulating effect of the autoclaved liver. A similar effect, due to B_1, was evident in the increased food consumption resulting from the partial substitution of yeast for liver in Groups 8, 9 and 10.

Although the factor in autoclaved liver may not be as necessary as the factor in yeast for efficient utilization of ingested food,[1] nevertheless, its effect on appetite was of importance in bringing about optimum growth on the purified diet. The experiments indicated that the influence of the antineuritic vitamin (B_1) on appetite may not be as specific as was suggested by Sherman and Sandels.[2] Burack and Cowgill[3] concluded from experiments on dogs that either the antineuritic vitamin was the sole appetite factor or another substance, not vitamin G (B_2), was required to supplement the antineuritic factor. Our own experiments emphasize the importance for normal appetite of *both* the antineuritic vitamin *and* another factor which is found in autoclaved liver.

5692

Effect of Splenectomy and of Other Surgical Procedures upon Circulating Blood Platelets (Rabbits).

P. E. STEINER AND F. D. GUNN.

From the Department of Pathology, Northwestern University Medical School.

It is a well established fact that splenectomy in the normal animal is usually followed by a considerable increase in the number of blood platelets. Largely on the basis of this fact the theory of the thrombolytic function of the spleen has been generally adopted. This theory was supported by the clinical observation of Kaznelson[1] that in purpura hemorrhagica, removal of the spleen was followed by an immediate increase of platelets and disappearance of purpuric hemorrhages. He assumed, therefore, that in this disease there was an overwhelming destruction of platelets in the spleen and suggested the name "splenogenous thrombolytic purpura." Most of the observational evidence in splenectomy in humans has supported Kaznelson's theory. Conclusions drawn from this kind of observation, however, are hazardous as there are so many unknown and uncontrollable factors involved.

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[1] Griffith, W. H., and Graham, C. E., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 862.

[2] Sherman, H. C., and Sandels, M. R., *J. Nutrition,* 1931, **3**, 395.

[3] Burack, E., and Cowgill, G. R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 750.

Moreover, it has been frequently observed that platelets are increased in number after other operations. The results of extensive clinical observations of this kind were published by Dawbarn, Earlam and Evans.[2] Bachman and Hultgren[3] concluded from experiments on rabbits that all operative procedures involving considerable trauma to tissues are followed by an increase in blood platelets but that the thrombocytosis induced by splenectomy is greater and of longer duration. Liles[4] observed that a group of rabbits, each of which had been the recipient of a transplanted spleen, showed an even greater increase in platelets than a group from which the spleens had been removed. On the tenth day the 2 groups showed the same platelet level but the level was sustained for a longer time in the splenectomized animals than in the recipients. He attributed the initial rise in both groups to traumatism.

The present experiments were carried out in an attempt to answer the question of the specificity of the effect of splenectomy upon blood platelets. Does removal of the spleen in the rabbit affect the platelet level to a greater degree than other operations involving a similar degree of trauma, or is the effect simply that of tissue injury in both instances?

The animals used were young rabbits weighing from 1390 to 2225 gm. at the time of the first operation. After a control period of a few days (usually 5) during which daily leukocyte, erythrocyte and platelet counts were made, the animals were prepared by clipping the hair and cleansing with green soap and water. Shaving and iodine were omitted because of the influence of skin irritation upon the platelets.[5] The pre-operative preparation and etherization were found to have no appreciable influence upon the platelet level. The technique for counting blood platelets has been described in previous publications.

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[1] Kaznelson, P., *Wien. klin. Wochenschr.*, 1916, **29**, 1451.

[2] Dawbarn, R. Y., Earlam, F., and Evans, W. H., *J. Path. and Bact.*, 1928, **31**, 833.

[3] Bachman, E. L., and Hultgren, G., *Compt. rend. Soc. de biol.*, 1926, **94**, 942.

[4] Liles, R. T., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 489.

Table I (A and B) shows the results of splenectomy in 13 previously unoperated animals. The spleen was delivered through an incision in the abdominal wall parallel to and just below the ribs. In the first 5 animals (Table I A) the pedicle of the spleen was clamped off with artery forceps before the spleen was cut away. Three of these 5 gave the highest platelet counts obtained in the entire series (No. 30, 164.8%; No. 18, 154.9%; No. 21, 106.9%). The average percentage increase for the 5 was 117.8%. In subsequent operations (8 rabbits) clamps were not used. The splenic vessels were ligated with silk and the spleen clipped away, with care to avoid injury to the pancreas. The average platelet increase for this sub-group (1 B) was 69.0%. The average increase for the entire group of 13 splenectomized rabbits was 87.8%.

TABLE I. Splenectomy.

A.

Rabbit No.	Wt. gm.	Platelets—thousands per cu. mm.				Post-op. day
		Normal average	Normal maximum	Experimental maxim.	Increase %	
18	1530	654	697	1,668	155	3
19	1540	908	958	1,652	82	2
21	2132	911	1,197	1,886	107	4
30	1760	570	578	1,512	165	3
31	1758	878	887	1,587	81	5
				Average	118	
B.						
36	1811	986	1,015	1,411	43	4
38	1754	806	888	1,381	65	4
41	1642	644	756	1,096	70	6
73	1465	646	691	984	52	4
75	2185	771	861	1,214	57	4
76	1955	704	828	1,392	98	4
77	1825	777	813	1,263	63	4
82	1560	760	842	1,546	103	5
				Average	69	

Table II shows the effect of control operations in 8 previously unoperated rabbits. In these operations an incision was made in the abdominal wall just as in the operations for splenectomy;

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[5] Steiner, P. E., and Gunn, F. D., *Arch. Path.*, 1931, **11**, 241.

TABLE II. Control Operation.

Rabbit No.	Wt. gm.	Platelets—thousands per cu. mm.				Post-op. day
		Normal average	Normal maximum	Experimental maxim.	Increase %	
74	2225	637	853	959	50	3
80	1665	685	730	1,127	64	4
81	1490	731	834	1,185	62	3
83	1440	526	677	1,104	110	5
85	1560	601	654	1,315	119	5
88	1630	767	799	881	15	4
89	1670	736	793	1,057	44	4
90	1380	568	625	863	52	3
				Average	64	

sutures were placed through the pedicle of the spleen and loosely tied, avoiding the inclusion of any blood vessels in the ligatures. In this group the average increase in platelets was 64.5% ; the greatest was 118.6% ; the least was 14.8%.

TABLE III. Splenectomy Followed by Control Operation.

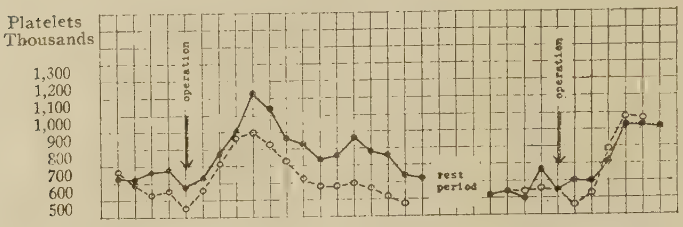
Rabbit No.	Splenectomy			Interval in weeks	Control Operation		
	Wt. gm.	% increase	Day of maximum		Wt. gm.	% increase	Day of maximum
73	1465	52	4	9	1890	34	4
75	2185	57	4	7	2670	112	6
77	1955	63	4	8	2855	30	4
82	1560	103	5	4	1805	93	4
	Average	68.9			Average	67.2	

In the third group of experiments (Table III) splenectomy was done and an average platelet increase of 68.9% was obtained. After an interval of from 4 to 9 weeks during which the blood picture remained practically normal, a control operation was done upon each of the 4 animals. These operations were followed by an average increase of 67.2%.

TABLE IV. Control Operation Followed by Splenectomy.

Rabbit No.	Control Operation			Interval in weeks	Splenectomy		
	Wt. gm.	% increase	Day of maximum		Wt. gm.	% increase	Day of maximum
74	2225	50	3	10	2610	24	4
80	1665	64	4	5	2045	83	4
81	1490	62	3	4	1755	29	4
83	1440	110	5	4	1705	105	5
	Average	71.8			Average	75.3	

Comparison of Effects of Splenectomy and Control Operation.



Solid line—splenectomy followed by control operation.
Broken line—control operation followed by splenectomy.
FIG. 1.

In the fourth group of rabbits (Table IV) the above procedures were reversed. The first, or control, operations yielded an average increase of platelets of 71.8%. The subsequent splenectomies gave 75.3% increase.

The results of the third and fourth groups of experiments just described are shown graphically in Fig. 1. The composite of the daily platelet counts of animals of group III, in which splenectomy preceded the control operation, is represented by a solid line. The composite of the counts of group IV, in which the control operation preceded splenectomy, is represented by a broken line. Although splenectomy in both cases gave a slightly higher average platelet level than the control operation, we do not regard the differences as significant, especially since the average normal in group III was higher than that of group IV.

During the first 4 groups of experiments it became increasingly apparent that the degree of thrombocytosis bore a definite relation to the amount of trauma sustained by the tissues. This theory was tested as follows: First, one of the rabbits of group III (No. 73) which had shown an increase of 52.3% of platelets after splenectomy and 33.7% after reincision and the placing of sutures in the peritoneum, was subjected to another "control" operation, designed to produce still greater trauma, namely, the crushing and removal of the end of the caecum through an 8 cm. midline incision. This was followed by an increase of 77.8%. Next, one rabbit was operated upon with the least possible trauma. A 4 cm. incision was made, the spleen was handled as gently as possible and sutures were placed in its peritoneal attachment. These were not tied. The spleen was allowed to drop back at once and the wound closed. In this case the increase of platelets was only 16.3% on the fourth postoperative day when the maximum was reached. On the fifth day the platelet level had already returned to normal. Eight days after the first operation a partial removal of the caecum was performed through an 8 cm. incision. The response to this operation was an increase of 82.0% on the fourth day.

Conclusions. Removal of the spleen in rabbits is followed constantly by an increase in the number of circulating blood platelets. Other operations involving a similar degree of trauma are followed by an increase of platelets which does not differ in time of occurrence, degree or duration from that observed after splenectomy. The degree of the rise depends upon the amount of trauma sustained by the tissues.

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Fate of Poliomyelitis Virus in the Brain After Intracerebral Inoculation of Normal and Convalescent Monkeys.*

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From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

The fate of poliomyelitis virus in the brain after intracerebral inoculation of normal monkeys has recently been studied very carefully by Fairbrother and Hurst.[1] These authors find the virus regularly present at the site of inoculation until the fifth day after infection, when the infectious agent suddenly disappears from the portal of entry on its way to further invasion of the more distant parts of the central nervous system. This critical point in the development of the disease, suggesting the operation of some autosterilizing mechanism in the infected brain tissue, apparently coincides with the first febrile response. The work of the above mentioned authors suggested a comparison of the survival of the virus after intracerebral inoculation in the normal monkey brain with the rate of disappearance from the site of inoculation in convalescent monkeys, immune through spontaneous recovery from the disease.

Six normal monkeys were inoculated intracerebrally with 1 cc. of a 10% virus cord emulsion, the potency of which was proved by simultaneous infection of several controls. By using a very

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* Under a grant from the International Committee for the Study of Infantile Paralysis, whose work is being financed by Jeremiah Milbank.

[1] Fairbrother, R. W., and Hurst, E. W., *J. Path. and Bact.*, 1930, **33**, 17.

TABLE I.

Survival of Poliomyelitis Virus in the Brain after Intracerebral Inoculation of Normal and Convalescent Monkeys.

Donor	Type of Animal	Interval, hrs.	Macroscopic Local Reaction	Rise of Temp. first 12 hrs. after inoculation	Recipient	Incubation Period, days	Symptoms	Pathological Examination or Reinfection
B56	Normal	12	No distinct lesion	0.8	B67	17	Ptosis of left upper eye lid	—
B57	”	24	No distinct lesion	0.8	B68	19	Complete paralysis Dead 22 dys	Typical Polio lesions
B22	”	48	No distinct lesion	1.0	B24	—	None Dead 40 dys	No typical Polio les.
B93	”	48	Sm. hemorrhagic area	1.0	B100	10	Tremor, awkward Dead 21 dys	Lesions suggestive of Polio in cervical and lumbar cord
B94	”	72	Sm. hemorrhagic area	0.8	C1	—	None	Reinfection Polio 18 dys
B96	”	96	Fairly large hemorrhagic area	1.2	C2	8	Complete paralysis Dead 15 dys	Typical Polio lesions
A53	Conv.	12	Slight inflammation	3	B4	—	None. Dead 6 mos later	No typical Polio lesions
B7	”	12	None	0	C9	—	None	Reinfection Polio 6 dys
90	”	24	Slight inflammation	2	B5	—	None Dead 21 dys	No typical Polio lesions
A89	”	24	None	0	C10	—	None Dead 25 dys	No typical Polio lesions
A22	”	48	Definite inflammation	2.8	B7	22	Almost complete paralysis	Used as Donor for C9
76	”	48	None	4	C11	12	Tremor, awkward	Reinfection Polio 7 dys
A91	”	72	”	5.4	C12	—	None	Reinfection Polio 8 dys
A95	”	72	”	4.2	C13	—	None Dead 29 dys	No typical Polio lesions
6	”	96	”	4	C14	—	None Dead 42 dys	No typical Polio lesions
86	”	96	”	2	C15	—	None	Reinfection Polio 7 dys

Controls: B59 (for B56, B57) Polio 6 days. B72 (for B93, B94, B96) Polio 8 days. B23 (for B22) Polio 9 days. C8 (for B7, A89, 76, A91, A95, 6, 86) Polio 10 days. B2 (for A53, 9, A22) Polio 9 days. C84 (for reinoculation of C1, C9, C11, C12, C15) Polio 6 days.

short needle and exercising special care so as to avoid deposition of the infectious material in the ventricle, the inoculated area could, in every instance, be accurately determined from the depth of the needle prick. At intervals varying from 12 to 96 hours the 6 monkeys were sacrificed and the sites of inoculation in each instance were transferred to a new passage animal by cerebral inoculation of the respective cortical area, emulsified into a 10% suspension. Another series of 10 convalescent monkeys was similarly infected together with an adequate number of controls, and, at the stated intervals, the respective sites of inoculation were likewise transferred to new monkeys. All of the convalescent monkeys except 3 (A89, A22, 76) showed definite residual paralysis at the time of the experiment. The results of the subinoculations in the 2 series are given in Table I.

It appears from Table I that in the normal monkey the virus may regularly be recovered from the site of inoculation during the first 24 hours of the infectious process, although the long incubation periods of Monkeys B67 and B57 indicate that only little growth can have occurred during that time. Between 48 and 72 hours, recovery of the virus is apparently less regular, none of the 3 monkeys sacrificed at these particular intervals harboring enough virus at the site of inoculation to cause typical poliomyelitis in the recipients. Beyond this point, i. e. at 96 hours after infection, a sufficient quantity of virus has apparently accumulated in the inoculated area to induce readily typical infection on passage. These findings are somewhat analogous to Levaditi and Nicolau's[2] observations with neurovaccinia virus in rabbits while in herpetic monkey infection the virus is apparently found more regularly at the site of inoculation, following a brief period of lag. (Levaditi and Lepine[3]).

The results obtained in the series of convalescent monkeys demonstrate clearly that at the early and late phases of the infection the injected virus is not present at the portal of entry in such a form as to cause typical poliomyelitis on passage. However, in 2 monkeys, sacrificed 48 hours after infection, the inoculated area evidently contained active virus in more or less virulent form. It is interesting to note that in the convalescent monkeys the virus was encountered at exactly that stage of the infection which seemed to be least favorable for the storage of the virus in the primarily infected monkeys, although the small number of experimental animals does not preclude chance differences between individual monkeys. We certainly do not consider the evidence sufficient as proving a fixation of the antigen in the immune tissues, such as has recently been found to be the case with rabies virus by Isabolinsky and Zeitlin.[4] A possibility of intervening specific and non-specific inhibitory factors must finally be kept in mind.[5] Without resorting to special physical and chemical treatment of the inoculum such as cataphoresis[6] and pH adjustment[7] this cannot be successfully eliminated.

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[2] Levaditi, C., and Nicolau, S., *C. R. Soc. Biol.*, 1922, **86**, 562.

[3] Levaditi, C., and Lepine, P., *C. R. Acad. Sciences*, 1929, **189**, 66.

In confirmation of our earlier work[8] it was found that, with the exception of 2 (B7, A89), all of the convalescent animals reacted within the first few hours after inoculation with a more or less marked rise of the body temperature, while none of the normal monkeys, during this time, displayed more than the usual traumatic febrile reaction. The 2 animals which failed to react showed extensive tuberculosis at autopsy.

A number of moneys, apparently having received with the transfer an amount of virus insufficient to cause typical poliomyelitis with definite paralysis, responded with an elevation of the body temperature, which occasionally seemed to show a tendency to run in distinct cycles, afebrile periods alternating with several days of high fever. Those cases may correspond with the abortive and relapsing infections recently described by Harmon, Shaughnessy and Gordon,[9] were it not for the fact that all of these monkeys succumbed to reinfection with fully developed symptoms of the disease.

The irregularity of the presence of the virus at the site of inoculation in the normal animal during the earlier intervals makes it difficult to establish positive proof for a quicker disappearance of the infectious agent from the brain of the convalescent animal. From the evidence at hand, however, it would seem that whereas in the normal animal the virus at the end of the fourth day after infection had considerably increased locally, the site of inoculation in the immune animal had been definitely sterilized.

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[4] Isabolinsky, M. P., and Zeitlin, A. J., *Z. f. Immun. Forschg.*, 1929, **62**, 233.

[5] Jungeblut, C. W., Meeting of Am. Soc. for Exp. Path., Montreal, 1931, abstract in print.

[6] Olitsky, P. K., Rhoades, C. P., and Long, P. H., *J. Exp. Med.*, 1929, **50**, 273.

[7] Sittenfield, M. J., Johnson, B. A., and Jobling, J. W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 517.

[8] Jungeblut, C. W., *J. Exp. Med.*, 1931, **53**, 159.

[9] Harmon, P. H., Shaughnessy, H. J., and Gordon, F. B., *J. Prev. Med.*, 1931, **5**, 139.

5694

Observations on Auricular and Ventricular Fibrillation.*

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Of the several theories which have been advanced to explain auricular and ventricular fibrillation 2 have been very widely accepted. One, advocated by Rothberger[1] and Scherf,[2] is that fibrillation is due to one or several foci sending out impulses at a very rapid rate, resulting in tachysystole which is manifested as fibrillation. The other theory, advanced chiefly by T. Lewis[3] and his coworkers, states that in auricular flutter and fibrillation a single mother wave circulates usually about the orifices of the superior and inferior vena cavae and sends off centrifugal waves into the remainder of the auricular tissue. Lewis and Garrey[4] suggest that auricular and ventricular fibrillation are the same and Lewis assumes that the mechanism in ventricular fibrillation is the same as was shown by him to be the cause of auricular fibrillation.

A series of experiments was performed by us to test the validity of the assumption that ventricular fibrillation is caused either by a single focus or a single mother wave as stated above. Twelve dogs were used, under morphine and sodium barbital anesthesia, and artificial respiration was maintained with the open chest. The heart was exposed and permitted to remain in a cradle made from the pericardium. Two direct non-polarizable leads were fixed to the left ventricle and 2 to the right. These were connected with an electrocardiograph. Ventricular fibrillation was induced by temporary faradic stimulation at the base of the right ventricle.

As soon as the rate of spontaneous fibrillation became somewhat stabilized a control tracing was made from the right and left ventricles, both of which were visibly fibrillating. The 2 ventricles were then quickly and completely severed from each other, care

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* Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

[1] Rothberger, C. J., *Klin. Wchnschr.*, 1922, **1**, 82; 1923, **2**, 1407.

[2] Scherf, D., *Z. f. d. ges. Exp. Med.*, 1928, **61**, 30.

[3] Lewis, T., Mechanism and Graphic Registration of Heart Beat, 1925, 2nd Edition, Chap. 31.

[4] Garrey, W. E., *Physiol. Rev.*, 1924, **4**, 215.

being taken not to displace them, and the records taken quickly again.

Separation of the 2 ventricles from each other should result in disappearance of fibrillation in one ventricle if the causative ectopic focus or mother ring were in the other ventricle. In any event the electrocardiogram after separation should be grossly changed. Such was not the case, except for slight slowing which could also be demonstrated in the intact fibrillating heart and was parallel in the 2 sides.

Another series of experiments was performed to test these theories in auricular fibrillation, using the same technique as in the ventricular records. Auricular fibrillation or flutter was induced by temporary faradic stimulation of the right auricle near the inferior vena cava. After the fibrillation persisted spontaneously for at least 30 seconds control records were made from the leads of each auricle. A heavy 10-inch clamp was then tighly placed along the inter-auricular septum up to the ventricles, crushing the tissue. Electrocardiograms were then taken from the leads of the right and left auricles. In the 2 experiments to date in which this was done, fibrillation persisted in the same form in both auricles, after crushing the tissue between them. These experiments cannot be explained by a single focus or single mother wave.

The results of our experiments are incompatible with the assumption of Lewis that a single mother circus wave with centrifugal offshoots is responsible for auricular or ventricular fibrillation. The same is true of the theory which supposes that a single focus sends out rapid impulses causing coordinated but very rapid contractions. Our results are not incompatible with the assumption that auricular and ventricular fibrillation may be due to numerous ectopic foci acting at the same time. The results of our experiments are also compatible with the theory of Garrey who assumes that an impulse becomes sinuous because of areas of transient block and that the impulse eventually gives rise to multiple, shuttling circuits which cause fibrillation.

SECRETARY'S ANNUAL REPORT

April 1, 1930 to April 1, 1931

Reduction of cost to members. The cost of excess space has been reduced from $4.20 to $3.50 per page. Beginning with the April number the rates were further reduced to $2.10 a page. This is considerably below cost.

The cost of cuts has been reduced 15 per cent.

MEMBERSHIP

Members, March 31, 1930	1017	
Elected during year	62	
Honorary Members	9	
Total		1088
Resignations, year ending March 31, 1931	12	
Deaths, year ending March 31, 1931	7	
Arrears, year ending March 31, 1931	7	26
Net Membership		1062

	1924	1925	1926	1927	1928	1929	1930	1931
Membership	627	692	779	851	936	986	1026	1062

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Subscriptions, March 31, 1931		397
Free Subscriptions, March 31, 1930	8	
Free Subscriptions, March 31, 1931		9
Exchange Subscriptions, March 31, 1930	8	
Exchange Subscriptions, March 31, 1931		9
Total, March 31, 1931		415
Total No. of PROCEEDINGS distributed 3/31/30		1419
Total No. of PROCEEDINGS distributed 3/31/31		1477

OFFICERS elected April 20, 1931

President: D. J. Edwards.
Vice-President: A. R. Dochez.
Secretary-Treasurer: A. J. Goldforb.
Council: W. J. MacNeal,
W. W. Palmer.

A. E. Cohn,
F. P. Gay,
W. J. V. Osterhout,
G. B. Wallace.
} hold over

Past Presidents (By constitution): E. B. Wilson, Simon Flexner, F. S. Lee, T. H. Morgan, James Ewing, Graham Lusk, W. J. Gies, Gary Calkins, G. B. Wallace, J. W. Jobling, S. R. Benedict, Peyton Rous.

Chairmen of Sections (See Sections).

Nominating Committee (elected April 20, 1931):

From New York: E. F. Dubois, Chairman; C. B. Davenport, A. F. Hess, A. M. Pappenheimer.

From Other Sections: H. M. Evans, R. Gesell, W. H. Howell, L. Loeb, A. B. Luckhardt.

Editorial Committee (appointed by Council): A. J. Carlson, B. M. Duggar, M. H. Jacobs, J. A. Kolmer, H. B. Lewis, W. deB. MacNider, A. M. Pappenheimer, A. J. Goldforb.

Membership Committee (appointed by Sections): W. R. Bloor, L. J. Cole, E. A. Doisy, C. W. Duval, R. A. Gortner, I. Greenwald, A. C. Ivy, E. G. Martin, G. H. Miller.

Financial Committee: W. J. Gies, W. H. Park, A. J. Goldforb.

PAST OFFICERS

Date	*President*	*Vice-Pres.*	*Secretary*	*Treasurer*
1903-04	S. J. Meltzer	W. H. Park	W. J. Gies	G. N. Calkins
1904-05	S. J. Meltzer	J. Ewing	W. J. Gies	G. N. Calkins
1905-06	E. B. Wilson	E. K. Dunham	W. J. Gies	G. N. Calkins
1906-07	S. Flexner	E. K. Dunham	W. J. Gies	G. N. Calkins
1907-08	S. Flexner	T. H. Morgan	W. J. Gies	G. N. Calkins
1908-09	F. S. Lee	T. H. Morgan	W. J. Gies	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie	G. Lusk
1910-11	T. H. Morgan	W. J. Gies	E. L. Opie	G. Lusk
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	G. Lusk
1912-13	J. Ewing	P. A. Levene	G. B. Wallace	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	C. Norris
1914-15	G. Lusk	W. J. Gies	H. C. Jackson	J. R. Murlin
				Secy.-Treas.
1915-16	G. Lusk	G. N. Calkins		H. C. Jackson
1916-17	J. Loeb	W. J. Gies		H. C. Jackson
1917-18	W. J. Gies	J. Auer		H. C. Jackson
1918-19	W. J. Gies	J. Auer		H. C. Jackson
1919-20	G. N. Calkins	G. B. Wallace		H. C. Jackson
1920-21	G. N. Calkins	G. B. Wallace		H. C. Jackson
1921-22	G. B. Wallace	J. W. Jobling		H. C. Jackson
1922-23	G. B. Wallace	J. W. Jobling		H. C. Jackson
1923-24	H. C. Jackson	J. W. Jobling		V. C. Myers
1924-25	H. C. Jackson	J. W. Jobling		A. J. Goldforb
1925-26	J. W. Jobling	S. R. Benedict		A. J. Goldforb
1926-27	J. W. Jobling	S. R. Benedict		A. J. Goldforb
1927-28	S. R. Benedict	P. Rous		A. J. Goldforb
1928-29	S. R. Benedict	P. Rous		A. J. Goldforb
1929-30	P. Rous	D. Marine		A. J. Goldforb
1930-31	P. Rous	D. J. Edwards		A. J. Goldforb

SECTIONS

Illinois

Chairman: F. C. Koch. Secretary: Lloyd Arnold. Members: 93.

Meetings: Billings Hospital, October 21, 1930.
University of Illinois, December 16, 1930.
Northwestern University Medical School, February 10, 1931.
Billings Hospital, May 26, 1931.

Iowa

Chairman: J. T. McClintock. Secretary: J. D. Boyd. Members: 29.
Meetings: State University of Iowa, November 6, 1930.
Iowa State College, December 4, 1930.
State University of Iowa, February 6, 1931.
State University of Iowa, April 30, 1931.

Minnesota

Chairman and Secretary: F. H. Scott. Members: 41.
Meetings: University of Minnesota Medical School, November 26, 1930.
University of Minnesota Medical School, January 28, 1931.
University of Minnesota Medical School, March 18, 1931.
University of Minnesota Medical School, May 20, 1931.

Missouri

Chairman: M. S. Fleisher. Secretary: D. Barr. Members: 46.
Meetings: Washington University School of Medicine, November 12, 1930.
St. Louis University School of Medicine, December 10, 1930.
Washington University School of Medicine, January 14, 1931.
Washington University School of Medicine, March 11, 1931.
St. Louis University School of Medicine, May 13, 1931.

New York

Chairman: D. J. Edwards. Secretary: A. J. Goldforb. Members: 662.
Meetings: New York Academy of Medicine, October 15, 1930.
New York Academy of Medicine, November 19, 1930.
New York Academy of Medicine, December 17, 1930.
New York Academy of Medicine, January 17, 1931.
New York Academy of Medicine, February 18, 1931.
New York Academy of Medicine, March 18, 1931.
New York Academy of Medicine, April 20, 1931.
New York Academy of Medicine, May 20, 1931.

Pacific Coast

Chairman: W. Ophüls. Secretary: T. D. Beckwith. Members: 78.
Meetings: University of California Medical School, October 15, 1930.
Stanford University, December 23, 1930.
Stanford University School of Medicine, February 18, 1931.
University of California, April 25, 1931.

Peiping (China)

Chairman: R. R. Hannon. Secretary: Shih-hao Liu. Members: 22.
Meetings: Peiping Union Medical College, October 30, 1930.
Peiping Union Medical College, March 26, 1931.

Southern

Chairman: C. C. Bass. Secretary: E. C. Faust. Members: 22.
Meetings: Tulane University, November 20, 1930.
Tulane University, February 14, 1931.
Tulane University, May 21, 1931.

Western New York

Chairman: W. O. Fenn. Secretary: E. F. Adolph. Members: 44.
Meetings: University of Rochester Medical School, October 18, 1930.
Syracuse University Medical School, December 13, 1930.
University of Rochester Medical School, February 28, 1931.
Clifton Springs Sanitarium, May 23, 1931.

Wisconsin

Chairman: L. J. Cole. Secretary: F. L. Hisaw. Members: 25.

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: E. J. Banzhaf, P. A. Fish, J. C. Hemmeter, C. C. Krumwiede, T. B. Robertson, Benjamin Roman, G. N. Stewart.

GIFTS

The Secretary wishes to acknowledge, with the thanks of the Council, the gift of back numbers from A. J. Carlson, B. B. Crohn, S. C. Harvey, W. J. V. Osterhout, I. K. Ragins, H. E. Robertson, C. A. Smith, L. von Meysenbug.

PROCEEDINGS

Average length of papers, 1.8 pp.
Preliminary manuscripts, 66%; complete manuscripts, 34%.

A. J. GOLDFORB,
Secretary.

TREASURER'S REPORT
April 1, 1930 to April 1, 1931

Surplus. The Treasurer is happy to report that, as in the past several years,* there is an annual surplus which amounts to $1,289.90.

RECEIPTS

Balance on hand, April 1, 1930		$ 3,411.68
Income, 1930-31:		
Dues	$ 4,215.58	
Reprints	2,744.09	
Excess Space	2,362.00	
Cuts	539.28	
Subscriptions	2,495.24	
Back Numbers Sold	493.03	
Bank Interest and Miscellaneous	65.81	
Total Annual Income		$12,915.03
Total Cash Available, April 1, 1930-April 1, 1931		$16,326.71

DISBURSEMENTS

Publication Cost of PROCEEDINGS:		
Printing	$ 5,376.22	
Reprints	3,035.00	
Cuts	493.62	
		$ 8,904.84
Administrative Expenses:		
Office Supplies, Postage and Telegrams	$ 527.79	
Salaries	2,080.00	
Storage and Insurance	82.69	
Miscellaneous	24.81	
Overpaid	5.00	
		$ 2,720.29
Total Annual Disbursements		$11,625.13
Surplus Transferred to Special Surplus Fund		900.00
Cash Balance, April 1, 1931		3,801.58
Total		$16,326.71

SUMMARY

Income (net)	$12,915.03
Disbursements (net)	11,625.13
Surplus for year	$ 1,289.90

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* For details see Treasurer's Report, Volume 27, 1930.

FUNDS

Endowment Fund

April 1, 1930	$12,006.42	
Contributions to April 1, 1931	33.00	
Interest to April 1, 1931	626.63	
Total		$12,666.05
Invested in N. Y. Title and Mortgage Co.	$ 6,000.00	
Invested in R. R. Coop. Bldg. and Loan	6,666.05	
		$12,666.05

Special Surplus Fund

April 1, 1930	$ 4,500.00	
Annual surplus transferred to Fund	900.00	
Interest to April 1, 1931	261.53	
Total		$ 5,661.53
Invested in Title Guarantee and Trust Co.	$ 5,400.00	
Invested in R. R. Coop. Bldg. and Loan Co.	261.53	
		$ 5,661.53

Life Membership Fund

Invested in R. R. Coop. Bldg. and Loan Co.		$ 75.00

MEMO

Bills payable	None
Bills receivable	$1,553.76

A. J. GOLDFORB,
Treasurer.

Auditors' Report

We have examined the Treasurer's report and verified the statements therein against the books of the Society. We desire to congratulate the Society upon the admirable manner in which the office of the Treasurer is conducted by Doctor Goldforb, with the assistance of Miss McCabe.

(Signed) REUBEN OTTENBERG,
HANS T. CLARKE.

April 10, 1931.

MEMBERS' LIST (Alphabetical)

HONORARY MEMBERS

Councilman, William T.	Harvard University
Halliburton, W. D.	London, England
Lombard, Warren P.	University of Michigan
Porter, William	Harvard University
Reichert, E. T.	University of Pennsylvania
Richet, Charles	Paris, France
Schaffer, E. S.	Edinburgh, Scotland
Von Muller, Friedrich	Munich, Germany
Welch, W. H.	Johns Hopkins University

MEMBERS

Abbott, Alexander C.	University of Pennsylvania
Abel, John J.	Johns Hopkins University
Adams, A. Elizabeth	Mount Holyoke College
Addis, Thomas	Medical School, Stanford University
Adler, Herman M.	Berkeley, California
Adolph, E. F.	University of Rochester Medical School
Albus, William R.	American Type Culture Collection, Chicago
Alexander, Harry L.	Washington University
Allen, Bennet M.	University of California
Allen, Edgar	University of Missouri
Allen, Ezra	N. Y. Homeopathic Medical College
Allen, William F.	University of Oregon
Alsberg, Carl L.	Stanford University
Alvarez, Walter C.	Mayo Clinic
Amberg, Samuel	Mayo Clinic
Amoss, Harold L.	Duke University Hospital
Anderson, John F.	E. R. Squibb & Son
Anderson, Rudolph J.	Yale University
Andrews, Edmund	University of Chicago
Anson, Mortimer L.	Rockefeller Institute, Princeton, N. J.
Arnold, Lloyd	University of Illinois
Aronson, J. D.	Henry Phipps Institute
Asher, Leon	Berne, Switzerland
Ashman, Richard	Vanderbilt University
Atchley, D. W.	Presbyterian Hospital, New York City
Atkinson, H. V.	Vermilion, S. D.
Atwell, Wayne J.	University of Buffalo, N. Y.
Aub, Joseph C.	Huntington Memorial Hospital, Boston
Auer, John	St. Louis University
Austin, J. Harold	University of Pennsylvania
Austin, W. C.	Loyola University
Avery, O. T.	Rockefeller Institute, N. Y. City

Bachem, Albert	University of Illinois Medical College
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Memorial Hospital, N. Y. City
Bailey, Cameron V.	N. Y. Post-Graduate Medical School
Baitsell, George A.	Yale University
Baldridge, Clarence W.	State University of Iowa
Baldwin, W. Manning	Albany Medical College
Ball, G. H.	University of California, Los Angeles
Balls, A. K.	Bremen, Germany
Banta, A. M.	Brown University
Barach, Alvin L.	College of Physicians and Surgeons
Barber, W. Howard	New York University Med. School
Barbour, Henry G.	University of Louisville
Bardeen, Charles R.	University of Wisconsin
Barlow, Orphus W.	Western Reserve University
Barnett, George D.	Stanford University
Barr, David P.	Washington University
Barron, E. S. G.	University of Chicago
Bass, Charles	Tulane University
Bast, T. H.	University of Wisconsin
Bauer, J. H.	Rockefeller Institute
Bauman, Louis	Presbyterian Hospital, N. Y. City
Baumann, E. J.	Montefiore Hospital, N. Y. City
Baumberger, J. Percy	Stanford University
Bayne-Jones, S.	University of Rochester
Bazett, H. C.	University of Pennsylvania
Beard, H. H.	Western Reserve University
Becker, E. R.	Iowa State College
Beckwith, T. D.	University of California
Behre, Jeannette A.	Cornell University Medical College
Belding, David L.	Boston University
Bellamy, Arthur W.	University of California, Los Angeles
Benedict, S. R.	Cornell University Medical College, N. Y. City
Benton, Anne G.	Vassar College
Berg, B. N.	Columbia University
Berg, William N.	Berg Biological Laboratory, N. Y. City
Bergeim, Olaf	University of Illinois
Bergey, David H.	University of Pennsylvania
Berglund, Hilding	University of Minnesota
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Beutner, R.	University of Louisville
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	University of Rochester
Bishop, George H.	Webster Groves, Mo.
Bishop, Katharine S.	University of California
Blackfan, K. D.	Harvard Medical School
Blair, John E.	Hospital for Joint Diseases, N. Y. City
Blake, F. G.	Yale University
Blakesley, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blatherwick, Norman R.	Metropolitan Life Insurance Co., New York City
Blau, Nathan F.	Cornell University Medical College
Blinks, L. R.	Rockefeller Institute
Bliss, Sidney	Tulane University
Bloom, William	University of Chicago

Bloomfield, A. L.	Stanford University Medical School
Bloor, W. R.	University of Rochester
Blum, Harold F.	University of California
Blumgart, H. L.	Beth Israel Hospital, Boston
Bodansky, A.	New York City
Bodansky, Meyer	University of Texas Medical College
Bodine, J. H.	State University of Iowa
Bodo, Richard	N. Y. University and Bellevue Med. College
Bollman, Jesse L.	Mayo Clinic
Boor, Alden K.	University of Chicago
Boothby, Walter M.	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.	Rockefeller Hospital
Bowen, B. D.	Buffalo General Hospital
Boyd, Julian D.	State University of Iowa
Boyd, Theo. E.	Loyola University
Boyden, E. A.	University of Minnesota Medical School
Bradley, H. C.	University of Wisconsin
Brand, Erwin	New York State Psychiatric Institute
Branham, Sara E.	Hygienic Laboratory, Washington, D. C.
Brewer, Robert K.	Syracuse University
Briggs, A. P.	St. Louis University
Bronfenbrenner, J.	Washington University
Bronk, D. W.	University of Pennsylvania
Brooks, Clyde	University of Alabama
Brooks, Harlow	New York University
Brooks, Matilda M.	University of California
Brooks, S. C.	University of California
Broun, G. O.	St. Mary's Infirmary, St. Louis
Brown, E. D.	University of Minnesota
Brown, J. Howard	Johns Hopkins University
Brown, John B.	Ohio State University
Brown, L. A.	George Washington University
Brown, Wade H.	Rockefeller Institute, N. Y. City
Buchanan, Robert E.	Iowa State College
Buchbinder, W. C.	Michael Reese Hospital, Chicago
Bulger, H. A.	Washington University
Bull, C. G.	Johns Hopkins University
Bullowa, J. G. M.	Harlem Hospital, N. Y. City
Bunting, C. H.	University of Wisconsin
Bunting, R. W.	University of Michigan
Burget, G. E.	University of Oregon Medical School
Burky, Earl L.	Johns Hopkins Hospital
Burnett, Theodore C.	University of California
Burns, Robert K., Jr.	University of Rochester
Burr, Harold S.	Yale University
Burrows, M. T.	Pasadena, Calif.
Byrne, Joseph	Fordham University

Calkins, Gary N.	Columbia University
Cannan, Robert K.	N. Y. University and Bellevue Med. College
Cannon, Paul R.	University of Chicago
Cannon, Walter B.	Harvard Medical School
Carlson, A. J.	University of Chicago
Carmichael, E. B.	University of Alabama School of Medicine
Carpenter, Charles M.	University of Rochester Medical School

Carruthers, Albert	Peiping Union Medical College
Carter, Edward P.	Johns Hopkins Hospital
Cash, James R.	Peiping Union Medical College, China
Castellani, Aldo	Tulane University
Cattell, McKeen	Cornell University Medical College
Caulfield, A. H.	University of Toronto
Cecil, R. L.	Cornell University Medical College
Chace, Arthur F.	N. Y. Post-Graduate Medical School
Chambers, Robert	New York University
Chambers, William H.	Cornell University Medical College
Chang, Hsi Chun	Peiping Union Medical College
Chang, Hsiao-Chien	Peiping Union Medical College
Cheer, S. N.	Peiping Union Medical College
Chen, K. K.	Eli Lilly and Co., Indianapolis
Chidester, F. E.	University of West Virginia
Child, C. M.	University of Chicago
Chittenden, R. H.	Yale University
Chou, T.	Peiping Union Medical College, China
Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	University of Michigan
Churchman, John W.	Cornell University Medical School
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	University of Wisconsin
Clarke, Hans T.	College of Physicians and Surgeons
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F.	Cornell University Medical College
Coghill, G. E.	Wistar Institute
Cohen, Barnett	Johns Hopkins Medical School
Cohen, Martin	N. Y. Post-Graduate Medical School
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Edwin J.	Harvard Medical School
Cohn, Isidore	New Orleans, La.
Cole, L. J.	University of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, William H.	Rutgers University
Coleman, Warren	New York University Medical College
Collens, William S.	Brooklyn Jewish Hospital
Collett, Mary E.	Western Reserve University
Collier, William D.	St. Louis University
Collins, H. H.	University of Pittsburgh
Collip, J. B.	McGill University
Conklin, E. G.	Princeton University
Connor, Charles L.	University of California Medical School
Cook, Donald H.	School of Tropical Medicine, San Juan, Porto Rico
Cook, S. F.	University of California
Cooke, J. V.	Washington University
Coombs, Helen C.	New York Homeopathic Medical College
Copenhaver, W. M.	Columbia University
Cori, Carl F.	State Institute of Malignant Diseases, Buffalo, N. Y.
Corley, Ralph C.	Purdue University
Corner, George W.	University of Rochester
Corper, H. J.	National Jewish Hospital, Denver, Colo.
Coulter, Calvin B.	Columbia University
Cowdry, E. V.	Washington University

Cowgill, George R.	Yale University
Crampton, C. Ward	N. Y. Post-Graduate Medical School
Crandall, L. A., Jr.	Northwestern University
Crile, George W.	Western Reserve University
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Crozier, W. J.	Harvard University
Cruickshank, E. W. H.	Dalhousie University, Halifax
Csonka, F. A.	U. S. Bureau Chemistry, Washington, D. C.
Cullen, Glenn E.	Vanderbilt University
Cummins, Harold	Tulane University
Cunningham, R. S.	Vanderbilt University
Curtis, G. M.	University of Chicago
Curtis, Maynie R.	Columbia University
Cushing, Harvey W.	Harvard Medical School
Cutler, Elliott C.	Western Reserve University

Dack, Gail M.	University of Chicago
Dakin, H. D.	Ossining, N. Y.
Danforth, Charles H.	Stanford University
Daniel, J. Frank	University of California
Daniels, Amy L.	University of Iowa
Daniels, Farrington	University of Wisconsin
Danzer, Charles S.	N. Y. Homeopathic Medical College
Davenport, C. B.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davies, H. Whitridge	The University, Sydney, Australia
Davis, D. J.	Wilmette, Illinois
Davis, Nelson C.	Rockefeller Foundation
Davison, Wilburt C.	Duke University
Dawson, James A.	College of the City of New York
Day, A. A.	Northwestern University Medical School
De Eds, Floyd	Stanford University Medical School
DeGraff, A. C.	N. Y. University Medical School
Derick, C. L.	Peter Bent Brigham Hospital, Boston
Detwiler, S. R.	Columbia University
Deuel, Harry J., Jr.	University of Southern California Medical School
Dickson, E. C.	Stanford University Medical School
Dienes, Louis	Massachusetts General Hospital, Boston
Dieuaide, Francis R.	Peiping Union Medical College, China
Doan, Charles A.	Ohio State University
Dochez, A. R.	Presbyterian Hospital, N. Y. City
Dock, William	Stanford University
Doisy, Edward A.	St. Louis University
Dolley, W. L., Jr.	University of Buffalo
Donaldson, H. H.	Wistar Institute, Philadelphia
Donaldson, J. C.	University of Pittsburgh
Dooley, M. S.	Syracuse University
Downs, W. G., Jr.	Yale Medical School
Drabkin, D. L.	University of Pennsylvania
Dragstedt, Carl A.	Northwestern University
Dragstedt, Lester R.	Northwestern University
Draper, George W.	Columbia University
Draper, John W.	New York City
Dresbach, M.	Albany Medical College
Dubin, Harry E.	Metz Laboratories, N. Y. City
DuBois, E. F.	Cornell University Medical College

Duggar, B. M.	University of Wisconsin
Dunn, L. C.	Columbia University
Dunn, Max	University of S. California
Durrant, Edwin P.	Ohio State University
Dutcher, R. Adams	Pennsylvania State College
Duval, C. W.	Tulane University
Dye, Joseph A.	Cornell University Medical College

Eberson, Frederick	University of California Medical School
Ecker, E. E.	Western Reserve University
Eckles, C. H.	University of Minnesota
Eckstein, Henry C.	University of Michigan
Eddy, Walter H.	Columbia University
Edmunds, C. W.	University of Michigan
Edwards, D. J.	Cornell University Medical College
Edwards, John G.	University of Buffalo
Eggston, Andrew A.	Manhattan Eye, Ear, Nose and Throat Hospital, N. Y. City
Eisberg, Harry B.	N. Y. University and Bellevue Medical College
Ellis, Max M.	University of Missouri
Elsberg, Charles A.	Neurological Institute, N. Y. City
Elser, W. J.	Cornell University Medical College
Emery, F. E.	University of Buffalo
Engle, E. T.	Columbia University
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erdmann, Rhoda	University of Berlin, Germany
Erlanger, Joseph	Washington University
Evans, Herbert M.	University of California
Ewing, James	Cornell University Medical College
Eyster, J. A. E.	University of Wisconsin

Faber, Harold K.	Stanford University Medical School
Fahr, George	University of Minnesota
Falk, I. S.	Washington, D. C.
Falk, K. George	Harriman Laboratories, N. Y. City
Falls, Frederick H.	University of Illinois
Famulener, L. W.	St. Luke's Hospital, N. Y. City
Farmer, Chester	Northwestern University
Faust, Ernest C.	Tulane University
Fenn, Wallace O.	University of Rochester Medical School
Ferry, R. M.	Harvard University
Field, Cyrus W.	New York City
Fine, M. S.	Battle Creek, Mich.
Fischer, Albert	Berlin, Germany
Fischer, Martin H.	University of Cincinnati
Fishberg, Ella H.	New York City
Fisher, Nelson F.	St. Luke's Hospital, Chicago
Fitch, C. P.	University of Minnesota
Fitz, Reginald	Peter Bent Brigham Hospital
Fitzgerald, J. G.	University of Toronto
Fleisher, Moyer S.	St. Louis University
Flexner, Simon	Rockefeller Institute, N. Y. City
Flinn, Frederick B.	Columbia University
Florence, Laura	New York Homeopathic Medical School

Forbes, Henry	Milton, Mass.
Forkner, Claude E.	Boston City Hospital
Fortuyn, A. B. D.	Peiping Union Medical College
Foster, Goodwin L.	Columbia University
Foster, Nellis B.	New York Hospital, N. Y. City
Frank, Robert T.	Mt. Sinai Hospital
Frazier, Chester N.	Peiping Union Medical College
Fred, E. B.	University of Wisconsin
Freedman, Louis	Metz Laboratory
Freeman, R. G., Jr.	Columbia University
Freund, Jules	Henry Phipps Institute, Philadelphia
Fridericia, L. S.	University of Copenhagen, Denmark
Frobisher, Martin, Jr.	Rio de Janiero, Brazil
Fry, Henry J.	New York University
Fulmer, Ellis I.	Iowa State College
Fulton, John F.	Yale Medical School
Funk, Casimir	Paris, France
Furth, Jacob	Henry Phipps Institute, Philadelphia

Gaebler, O. H.	Henry Ford Hospital, Detroit
Gager, C. Stuart	Brooklyn Botanic Garden, Brooklyn, N. Y.
Gahl, Rudolph	University of California
Gamble, James L.	Harvard University
Garbat, Abraham L.	Lenox Hill Hospital, N. Y. City
Gardner, Leroy U.	Saranac Lab. for Study of Tuberculosis
Garrey, Walter E.	Vanderbilt University
Gaskell, John F.	Cambridge, England
Gasser, Herbert S.	Washington University
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Shear, M. J.	Harvard Medical School
Sheard, Charles	Mayo Clinic
Shelling, D. H.	Johns Hopkins Hospital
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Sherman, Hartley E.	Bureau of Science, Manila, P. I.
Sherman, James M.	Cornell University
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Shevky, Eshref	Bolinas, California
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Shope, R. E.	Rockefeller Institute, Princeton, N. J.
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Sperry, Warren	Babies Hospital, N. Y. City
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